ISOLATION AND CHARACTERISATION OF BACTERIAL PATHOGENS IN ORTHOPEDIC IMPLANT ASSOCIATED INFECTIONS IN A TERTIARY CARE CENTRE

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In

MICROBIOLOGY – BRANCH IV

The Tamil Nadu

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CERTIFICATE

This is to certify that the enclosed work **"ISOLATION AND CHARACTERISATION OF BACTERIAL PATHOGENS IN ORTHOPEDIC IMPLANT ASSOCIATED INFECTIONS IN A TERTIARY CARE CENTRE"** submitted by **Dr. K.Sivaram** to The Tamilnadu Dr. MGR Medical University is based on bonafide cases studied and analysed by the candidate in the Department of Microbiology, Coimbatore Medical College and Hospital , Coimbatore during the period from July 2016 to June 2017 under the guidance and supervision of **Dr. N.Mythily, MD.,** Professor and Head of Department , Department of Microbiology and the conclusion reached in this study are his own.

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I, **Dr. K.Sivaram,** solemnly declare that the dissertation entitled **"ISOLATION AND CHARACTERISATION OF BACTERIAL PATHOGENS IN ORTHOPEDIC IMPLANT ASSOCIATED INFECTIONS IN A TERTIARY CARE CENTRE**" was done by me at Coimbatore Medical College Hospital, Coimbatore during the period from July 2016 to June 2017 under the guidance and supervision of **Dr.N.Mythily, M.D.,** Professor & HOD, Department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr. MGR. Medical University towards the partial fulfilment of the requirement for the award of M.D. Degree (Branch – IV) in Microbiology.

I have not submitted this dissertation on my previous occasion to any University for the award of any degree.

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CONTENTS

CONTENTS

LIST OF COLOUR PLATES

S.NO	COLOUR PLATES
1	Gram Stain - Gram positive cocci in clusters of Staphylococcus aureus.
2	Nutrient Agar – Golden yellow colonies of Staphylococcus aureus.
3	Beta hemolytic colonies of Staphylococcus aureus in 5% sheep blood agar.
4a	Tube coagulase test
4b	Mannitol Salt Agar– Yellow colonies of Staphylococcus aureus.
5a	Direct Gram stain showing pus cells and Gram negative bacilli
5b	Gram stain showing Gram negative bacilli
6	MacConkey Agar: Mucoid Lactose Fermenting Colonies of Klebsiella
7	MacConkey Agar – Flat lactose fermenting colonies of E.coli
8	Nutrient agar –Pyocyanin Pigmentation of Pseudomonas aeruginosa
9	Blood Agar Plate Swarming growth of Proteus
10	Biochemical reactions of Pseudomonas aeruginosa
11	Antibiotic Sensitivity of Staphylococcus aureus by disc diffusion method
12	E Strip showing Vancomycin sensitivity of less than 2µg/ml for Staphylococcus aureus
13	Antibiotic susceptibility of S.aureus resistant to Cefoxitin (MRSA)
14	Inducible Clindamycin resistance D-Test
15	Agarose gel electrophoresis of PCR
16	Phenotypic screening of ESBL by using 4 drugs
17	Phenotypic confirmation of ESBL by combined disc method
18	Implant – External Fixator
19	Implant – Dynamic Compression Plate

LIST OF TABLES

LIST OF CHARTS

LIST OF ABBREVIATIONS

SSIs	- Surgical Site Infections
BAP	- Blood Agar Plate
MAC	- MacConkey Agar Plate
MSA	- Mannitol Salt Agar
RCM	- Robertson Cooked Meat Media
CoNS	- Coagulase Negative Staphylococcus
E.coli	- Escherichia Coli
RTA	- Road Traffic Accident
UTI	- Urinary Tract Infections
CTX	- Cefotaxime
MRP	- Meropenem
CLSI	- Central Laboratory Standard Institute
PCR	- Polymerase chain Reaction
LPS	- Lipo Poly Saccharide
PBP	- Penicillin Binding Protein
MIC	- Minimum Inhibitory Concentration
Mm	- Millimeter
µg	- Microgram

MDR	- Multi-drug Resistant
MRSA	- Methycillin Resistant Staphylococcus aureus
MSSA	- Methycillin Sensitive Staphylococcus aureus
ESBL	- Extended Spectrum Beta Lactamase
ODRI	- Orthopedic Device Related Infection
PJI	- Prosthetic Joint Infection
CDC	- Center for Disease Control

INTRODUCTION

INTRODUCTION

In the modern era, orthopaedic implants have revolutionized the treatment of bone fractures and non infectious arthritis. Today implant surgery has become one of the commonest orthopaedic operations, because of the success of this procedure in restoring function of bones and joints. This is the major procedure to alleviate pain and improve mobility in people with bone fracture and damaged joints.

Less than 10% of prosthesis recipients develop implant-associated complications during their lifetime, predominantly implant failure. However, the absolute number of patients with infection continuously increases as the number of patients requiring such implants grows.

Implant failure are of two types, loosening from joint infection is referred to as septic failure, loosening from non infectious reasons such as bone fracture, brittle bones that cannot hold the implant or some other mechanical problem is called aseptic (without infection) failure.

In the UK and USA, about 800000 joint arthroplasties are done annually, with projections to greater than 4 million by 2030[3]. The incidence of infection following elective orthopaedic surgery is in the range of 0.7% to 4.2%, while incidence can be much higher in trauma cases where infection rates range from 1% of closed fractures to more than 30% in open fractures[7]. It is a challenging task to treat ODRI

which may lead to implant replacement, and in severe cases amputation and even mortality.

Definition:

Orthopaedic implants can be defined [1] as medical devices used to replace or provide fixation of bone or to replace articulating surface of a joint. In simpler words orthopaedic implants are used to either assist or replace damaged or troubled bones and joints. Orthopaedic implants are mainly made from stainless steel and titanium alloys for strength and lined with plastic to act as artificial cartilage in order to reduce the stress at the articulating surfaces. Some implants are cemented into place and others are pressed to fit so that the bone can grow into the implant for strength.

Some examples of orthopaedic implants [1] are, a) Orthopaedic plates b) Orthopaedic nails c) Orthopaedic screws

The key factor that guides bone healing is the interfragmentary movement, which determines the tissue strain and consequently the cellular reaction in the fracture healing zone. Thus the methods of fracture fixation will be evaluated by considering their ability to reduce the interfragmentary movement.[1].

Classification of orthopaedic fixation devices.[1]

1)Internal fixation devices: Screws, plates, wires and pins, intramedullary rods & nails, and spinal fixation devices.

2) External fixation devices: a) Fracture fixation devices- Radius, Tibia, Pelvis. b) Bone lengthening- IIizarov device

Classification of Orthopaedic Device Related Infections(ODRI)

Definition:

An implant associated infection is defined as a host immune response to one or more microbial pathogens on an indwelling implant[12].

Orthopaedic device related infections occur per operatively by bacterial contamination of the surgical site during surgery or immediately thereafter. It could be a haematogenous microbial spread through blood from a distant focus of infection or contiguously by direct or lymphatic spread from an adjacent infectious focus or by penetrating trauma.

Classification of ODRI:

Device related infections can be classified as defined by the "**Centres for disease Control and Prevention**" into three categories based on duration and timing of appearance of symptoms post surgery

1) **Early post operative infection**: These occur in the immediate post operative period within 2 to 4 weeks. It is caused by virulent organisms like Staphylococcus aureus and E coli.
2) **Late/chronic infection**: Manifestation of infection occurs after 1 month of surgery. Mostly they are organisms of low virulence for example CoNS (Coagulative negative staphylococcus species), Propionibacterium acne.
3) **Haematogenous infection:** Manifestation of infection occurs after 2 years of surgery. Haematogenous seeding may be triggered by skin, dental, respiratory, and urinary tract infections.

Sources of infectious pathogens[9] which facilitates device related infections include environment of the operating room, surgical equipments, clothing worn by medical and paramedical staffs, resident bacterial flora on the patients skin etc.

Bacteria exists in two forms: free floating(planktonic) and surface associated(biofilm)[12]. Implant associated infection are the result of bacterial adhesion to an implant surface and subsequent biofilm formation at the implant site. Biofilms mainly confer resistance to many antibiotics and impair host immune response. As a result standard antibiotic therapy mainly reverses signs and symptoms caused by planktonic bacteria released from biofilm but fail to kill bacteria in the biofilm.

So once frank implant associated infection develops, the management becomes difficult and comprises mainly of repeated surgical debridement at frequent intervals. It is not only a physical ailment for the patient but also adds to his psychological and financial load.

Following are the bacterial pathogens encountered in the orthopaedic implant infections[11]. Staphylococcus aureus is the most frequently isolated organism followed by Klebsiella spp, Pseudomonas aeroginosa, Proteus spp, and also polymicrobial. About 50 to 60% of these infections develop within 1 month of surgery, 30 to 40% develop within 2yrs, and the remainder develop more than 2yrs after surgery.

Each hospital has its own unique bacterial flora to which patients are at risk for acquiring infection during hospitalization. In such situations microorganisms exhibit unique pattern of antibacterial activity during a certain period. Since initial antibiotic therapy is empirical it is important to know the prevailing antibiotic susceptibility pattern of individuals by routine surveillance. Hence a regular bacteriological review of infected implants are therefore necessary if affected patients are to receive quality health care.

Infections associated with implants represent one of the most devastating complications associated with high morbidity and substantial cost. In the recent years the organisms isolated from these infected cases are showing increased resistance to commonly used first line antibiotics

and multidrug resistance. So early detection of implant infection is therefore crucial. The advantage of which include timely administration of antibiotic prophylaxis, strict infection control practices, and formulating an effective antibiotic policy to prevent and treat ODRI in patients, and thereby reduce the spread of antibiotic resistant strains so as to improve the clinical outcome among the patients.

AIM & OBJECTIVES

AIM AND OBJECTIVES

AIM:

To study and characterise the bacterial pathogens and their current antibiotic susceptibility/resistance pattern in patients with orthopaedic implant associated infections.

OBJECTIVES:

1) To isolate and identify the bacterial pathogens in patients with orthopaedic implant associated infections.
2) To assess the antibiotic susceptibility pattern of microbial isolates.
3) To determine the resistance pattern among the isolates.
4) Genotyping of the resistant strains.
5) To formulate antibiotic policies and proper infection control measures with reference to implant associated infections in our hospital.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Hall marks in the history of orthopaedic implants for trauma and joint replacement.

Nowadays implant surgery has become one of the commonest sugery, because of the success of this procedure in restoring function to the affected bone and joint. In this historical review, an attempt was made to present the evolution of orthopaedic implants for trauma and arthroplasty during the 20th century.

Trauma, the early era

It was during the 1860s, that the first aseptic surgical technique had been described by the British Surgeon Joseph Lister(1827-1912). At the beginning of the 20th century attempts to use implants in orthopaedic surgery were still scarce and relatively rare as medical community had not been persuaded for the use of artificial parts, metallic or non metallic.

The first attempts were related to the reconstruction of fracture of long bones and their joints. Sir William Arbuthnot Lane(1856-1943), British Surgeon together with orthopaedic nurse British Dame Agnes Gwendoline Hunt(1866-1948) and Belgian surgeon Albin Lambotte(1866-1955) designed a fracture plate made of stainless steel.

The post Lister era, was marked by the fact that implants and biomaterials could not be correlated to success or failure of prosthetics

and it was a common practice to remove an implant soon after its original purpose of use was achieved. Even though newer materials were manufactured, the use of stainless steel remained extensive. Martin Kirschner (1879-1942) a German surgeon, used pins and wire to reinforce the connection of the implant to the bone, a method that carries his name ever since, K-wire or Kirschner wire.

Surgical treatment of the fracture of the femoral neck was the next achievement in implant surgery. During 1926,Ernest William Hey Groves(1872-1944) first used common carpentry screws, which was later replaced by Vitallium.

The intramedullary nailing was the field for research by Ernest Hay Groves, He was the one who used metallic rods first. He had noticed that the fracture healed without the use of plaster or a traction device, and it was a breakthrough in the history of Orthopaedics.

External fixation methods were also developed, in the U.S. Clayton Parkhill(1860-1902) from Denver, had presented a paper on external fixation device to the American College of Surgeons in 1897 and his method was extremely popular during the 1st World War.

Trauma in the post war era

In the years that followed 2nd World War, Orthopaedic Surgeons tried to perfect the methods of fracture fixation mainly by using wires and pins as developed by German Surgeon Martin Kirschner(1879-1942). A new boost was given by the advent of antibiotics, a fact that made possible prolonged operations with a reduced risk for infection.

Nowadays external fixation is still in use for the treatment of fractures with significant soft tissue damage for immobilization of pelvic and long bone fractures especially in multi trauma patients. Disadvantages were considered to be limited joint flexion , the increased risk of non-union and the infection in the pin entry sites (10% of cases).On the other hand, replacing an external fixator with an intramedullay nail, also increases the risk of infection.

The introduction of antibiotics was a major step in the treatment of infections. Alexander Fleming in 1928 made a discovery that the fungus Penicillum produced a substance that could destroy staphylococcus.

The active microbial substance was used clinically by Howard Foley in 1940 to treat a severe mixed infection with staphylococcus aureus and streptococci in Oxford. Penicillin was rapidly introduced in clinical practice followed by streptomycin in 1944 and other numerous antibiotics.

John Burke published his study about the timing of chemoprophylaxis. During his study he observed that antibiotics given systemically were effective against staphylococcal strains, only if present within short period of incision. These data led to universal agreement that adequate systemic antibiotics need to be present in the immediate pre incision period to ensure maximum effectiveness.

Trauma, biological osteosynthesis and contempory developments

During the modern era 1990, new systems made of bioabsorbable non-metallic materials became popular. The greatest advantage of these implants was that they are progressively absorbed and do not remain in situ like those that are metallic , and therefore a second operation for the removal of materials after the fracture healing is avoided.

Definition:

Orthopaedic implants can be defined[1]as medical devices used to replace or provide fixation of bone or to replace articulating surface of a joint. In simpler words, orthopaedic implants are used to either assist or replace damaged or troubled bones and joints. Orthopaedic implants are mainly made of stainless steel and titanium alloys for strength and lined with plastic to act as artificial cartilage in order to reduce the stress at the articulating surfaces.

Some examples of orthopaedic implants[1]are orthopaedic plates, orthopaedic nails, orthopaedic screws. The key factor that guides bone

healing is the interfragmentary movement, which determines the tissue strain and consequently the cellular reaction in the fracture healing zone.

Classification of orthopaedic fixation devices.[1]

1) Internal fixation devices:

Screws, plates, wires and pins, intramedullary rods & nails, and spinal fixation devices.

2) External fixation devices:

a) Fracture fixation devices- Radius,Tibia,Pelvis. b) Bone lengthening- IIizarov device.

Incidence and prevalence of ODRI

Orthopaedic and trauma device-related infection(ODRI) remains a major complication in modern trauma and orthopaedic surgery. The global incidence of ODRI remains approximately 1% after primary and 3% to4% after revision surgery.

Lakshminarayana.S.A,et al.,in 2015 reported an incidence rate of implant infection varying from0.5%to2% in fixation of closed fractures to 30% after fixation of open fractures. This variation was due to the differences in clinical procedures, types of organisms, resistance pattern of the organisms, control measures, and hospital environment.

Muhammad Shoaib Khan et al., (2007) in his study reported the rate of infection as 5.76% which was higher than the accepted standard which should be <1% and this was due to predisposing factors like advanced age, prolonged surgery time, smoking, and skin abrasion.

Prathab et al., in (2015) study had showed culture positivity of 73% which is less when compared to other studies whereas Anisha Fernandez et al reported 84%, and Khosravi et al, and Vishwajith et al, reported the culture positivity of 93% and 94% respectively. Gomez et al, reported even lesser positivity of 60%.

Ravikanth et al, in 2015, found out the rate of implant infection was 12%, as compared to study by Maksimovic et al, which showed an infection rate of 22% which is much higher

In a study of 165 patients by Khosravi et al, (2009), the incidence of implant infection in open and external fractures,was 17.6%, and 82.4% of implant infections occurred in closed and internal fractures.

Risk factors

Major risk factor for development of implant associated infection depends upon the extent of damage to the soft tissue and periosteum following fracture. Devascularised bone and other necrotic tissue are ideal for multiplication of bacteria. Damage to the periosteal blood supply and lack of perfusion of the soft tissues not only interfere with fracture healing and but also prevent humoral and immunological host defence

mechanisms from reaching the traumatised area, and fighting the spread and multiplication of inoculated microorganisms at the bone- implant interface.

The type of fracture whether it is open or closed fracture, type of implant used, type of surgery whether emergency or elective procedure, choice of antibiotic prophylaxis, duration of surgery, type of anaesthesia, duration of hospital stay, all these factors influence the risk of developing implant infections.

In a prospective study of 63 patients by Prathab et al (2015) the most common co morbid condition/risk factor associated with ODRI was found to be old age, followed by Diabetes mellitus, and H1N1 in 5 cases.

A six years of study by Dabet Rynga et al (2016), observed that the most probable sources of infection were from the stretchers, the stands for intravenous solutions, the OT table, instrument trolley, X-ray machine, suction cannula tip. The other sources of infection are also described by Aggarwal et al, who observed that the source of infection was most probably the gut flora, which in the prolonged bed ridden patients can contaminate their surroundings.

Patient related risk factor:

Age: The risk of ODRI is found to be higher in older patients. In comparision to younger population these patients are usually characterised by impaired immune response to infectious agents, inferior nutritional status, and possibly more co-morbidity.

Co-morbid conditions: it is well established that in patients undergoing surgery Diabetes mellitus is associated with increased risk for complications and increased length of hospital stay. In general hyperglycaemia may cause distruption of the host response to a bacterial load.

Connective tissue disorders: Connective tissue disease has been correlated with increased risk of ODRI. This group of conditions including Rheumatoid arthritis, Systemic lupus erythematosis, Psoriatic arthritis is associated with immune modulation resulting in predisposition to infection. More over patients on chronic immunosuppressive and glucocorticoid treatment has been identified as a risk factor for infection following implant surgery. Novel biologic agents (TNF α blockers)used in the treatment many of these conditions are known to adversely affect the patient's ability to fight infection are additional risk factors.

Malignancy: The presence of malignancy has been confirmed to increase ODRI. It might be due to the potential effects of malignancy associated treatment such as glucocorticoids and cytotoxic agents.

Other conditions include preoperative anaemia, liver disease, chronic kidney disease, previous myocardial infarction, congestive heart failure, pulmonary diseases, concurrent urinary tract infection, cigarette smoking, alcohol abuse etc.

Pathogenesis and clinical presentation of implant infections

A fundamental concept in the pathogenesis of ODRI is the formation of biofilms by the infecting organism. An understanding of pathogenesis of biofilm formation facilitates optimal diagnosis and treatment. All implants undergo physiological changes after implantation. The earliest and clinically the most important step is the "race for the surface", a contest between tissue cell integration and bacterial adhesion to the same surface[11].

Biofilm

Biofilm is a colony of microorganisms suspended within a self produced matrix, the extracellular polymeric substance. Estimates report that 99% of bacteria can exist within a biofilm state. The common organisms responsible for biofilm associated infection include Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeroginosa.[13]

Biofilm formation

The adhesion of a planktonic bacterium to an inert surface leads to the formation of a biofilm in phases:

adhesion→aggregation→mutation→dispersal. The process depends upon local conditions such as hydrophobicity and acidity, oxygen concentration, presence of inert material and the ability of the bacterium to initiate contact via pili/flagella.

Biofilm function

The ultimate function is to create a protective environment within which bacteria can sustain a state of existence without overwhelming the host. Secretion of virulence factors conveys a unique environment for the bacteria to continue to evolve and mature allowing evasion of innate and adaptive immune responses and development of tolerance to antimicrobial agents.

Clinical presentation:

Infections associated with 'internal fixation devices' is multifaceted [12] and depends on 1)the preceding trauma or surgical procedures, 2)the anatomical localization, 3)the quality of bone and surrounding soft tissue, 4)the interval between microbial inoculation and manifestation of infection and5)the type of organism.

Most infections associated with internal fixation devices are acquired exogenously, typically in the post operative period. Early post operative infection (<3weeks) is characterized by erythema, local hyperthermia, protracted wound healing and a secreting wet wound. Thus wound healing disturbances after internal fixation are highly suspicious of early infection

and should be managed as such. The first step is always debridement surgery for diagnostic and therapeutic purposes.

Delayed (3-10 weeks) or chronic (>10 weeks) infections are typically due to low virulence microorganisms such as coagulase-negative staphylococci or result from inadequate treatment of early infection. Delayed or chronic infections manifest as persistant pain, or signs of local inflammation such as erythema, swelling or intermittent drainage of pus. Radiologically delayed consolidation, pseudarthrosis, bone sequesters and soft tissue calcification can be observed.

'External fixation' is a surgical treatment used to stabilize bone and soft tissues at a distance from the operative or injury site. With external fixation pins are inserted through the skin into the bone and held in place by an external frame. The usual indications are open fractures such as tibia fracture which requires dressings or attention to a wound or flap. Without proper technique for pin insertion and meticulous pin tract care, this may result in pin sepsis, occurring in 30% of patients. It varies from minor inflammation to superficial infection requiring antibiotics, local wound care, and occasional pin removal , to osteomyelitis requiring sequestrectomy.

Causes of pin sepsis are:[13]

1)Site selection: the more the soft tissue is there , the greater is the chance of sepsis

2)Skin tethering: close wounds, if possible before inserting the pin, as closure will be

to relieve skin tension.

3)Use of power instruments: drilling wide diameter pins directly into the bone will generate heat, this may lead to sequestrum formation and sepsis.

4)Pin care: inadequate pin care and poor hygiene may lead to sepsis.

Novel classification of periprosthetic joint infection(PJI) [12]

Type of PJI	**Characteristics**
1)Acute haematogenous	-Infection with a duration of symptoms of 3 weeks or less after an uneventful post operative period
2)Early postinterventional	-Infection that manifests within 1 month after an invasive procedure such as surgery or arthocentesis
3)Chronic	-Infection with symptoms that persists for more than 3 weeks, beyond the early post interventional period

Diagnosis of implant infections[5]

A combination of clinical, laboratory, histopathology, microbiology and imaging studies are required for the diagnosis of implant infection.

Early infection is usually easy to diagnose, as it present's clinically with both local and systemic signs. There may be erythema, edema, induration, raised temperature or even frank pus discharge at the operative site. Systemic signs include fever, malaise, lethargy, and loss of appetite. Secretory wet wounds and unsatisfactory wound healing are common presentations.

In case of delayed and late infections, a high degree of suspicion is required. Delayed or chronic infection may present as persistent pain at local site, local signs of inflammation or sinus. It is often seen that sinuses/discharge from interlock screw/bolts or persisting discharging sinus in a plated fracture. Unusual delay in fracture healing, loosening of plates and screws should be taken as highly suspicious in favour of infection even if there may not be exterior signs of infection.

Radiography:

1) Plain X-ray of the operative site may not contribute to the diagnosis in the initial post operative days. If infection is suspected clinically, even in the absence of radiological signs of infection , it is wiser to explore the wound and send the material for histology, culture and sensitivity.

2) Contrast arthrography improves accuracy of assessing implant stability. Synovial outpouchings and abscesses are typical signs of infection.[4]

Ultrasonogram:

Ultrasonography may detect fluid effusions around the prosthesis and can be used to guide joint aspiration and drainage procedures.[4]

Nuclear medicine: Scintigraphy by means of a technetium(Tc99m) scan, Galium citrate(Ga67), or indium(In111)-labelled leukocyte scan may be helpful in the diagnosis of ODRI. However this approach is expensive, and accuracy of these methods is still limited.[5]:

Haematology:

Blood leukocyte counts and differential counts may show leukocytosis and neutrophilia in acutely acquired infections. Post surgery, C- Reactive protein and ESR is acutely elevated and returns to normal within weeks. Therefore preoperative and repetitive postoperative values are more informative than a single value in the post period. In prosthetic knee infections synovial fluid leukocyte count of more than 1.7 x 109/l and differential of more than 65% neutrophils has a sensitivity for infection of 94% and 97% and specificity of 88% and 98% respectively.[5]

Histopathology:

Histopathological examination of the periprosthetic tissue has a sensitivity of more than 80% and a specificity of more than 90%.

Histopathology examination does not identify the causative organism but it can confirm the presence of infection.

Sonication:

New diagnostic approaches include sonication of removed implants to dislodge adherent microorganisms growing in biofilms and the use of molecular techniques to improve diagnostic yield.[5]

Bacterial pathogens in implant infections

The following are the organisms isolated from orthopaedic implant infections. Staphylococcus aureus is the most common organism, followed by Klebsiella spp, Pseudomonas aeruginosa, Proteus spp, Coagulative negative Staphylococcus, Escherichia coli, Acinetobacter spp, and Enterococcus fecalis .

Staphylococcal infections:

Staphylococcus aureus is the most virulent organism among the Staphylococcus species and remains a major cause of infection in implants. It is a pluripotent pathogen causing disease by both toxin mediated and non toxin mediated mechanisms. Staphylococcus aureus is responsible for not only implant infections but also causes life threatening systemic infections. The less virulent Staphylococci- Coagulase negative staphylococci(CONS) are part of normal skin flora but act as opportunistic pathogens occasionally.

The genus Staphylococcus belongs to family Micrococcaceae and contains 36 species. It is an aerobe and facultative anaerobe, non-motile,

Gram positive cocci about 1µm arranged in clusters, pairs and tetrads. Staphylococcus produce catalase and ferments many sugars and forms lactic acid but no gas. Each strain varies by its proteolytic activity and they produce many extracellular substances. Staphylococci can survive in dried items, tolerate heat(up to 50°C for 30 minutes) and 9% sodium chloride.

Virulence factors of staphylococcus aureus:

1)Peptidoglycan and teichoic acids:

The cell wall of Staphylococcus aureus contains peptidoglycan and teichoic acid a unique rebitol phosphate polymer. The main function of teichoic acid is to help the bacteria to adhere to mucosal surface and peptidoglycan provide rigidity to cell wall and contributes to virulence by activation of complement, enhancement of chemotaxis of polymorph nuclear cells and interlukin-1 production by monocytes.

Cell surface adhesions:

fibrinonectin binding proteins, collagen binding proteins, and clumping factor.

ll) Protein A:

Staphylococcus aureus cell wall contains specialized protein on its cell surface called protein A; they belong to group of adhesins called microbial surface components recognizing adhesive matrix molecules (MSCRAMMS). Protein A has many biological properties, such as

anticomplimentry, chemotactic, mitogenic, inhibition of opsonisation and induction of platelet damage. It mediates co-agglutination reaction.

lll) Capsular polysaccharide:

Some strains of Staphylococcus aureus produce exopolysaccharide that prevents them from ingestion by polymorphonuclear cells and there are 11 types of capsular polysaccharide.

lV) Enzymes:

Many enzymes are produced by Staphylococcus aureus

The enzyme catalase produced by Staphylococcus aureus inactivates the free radicles and hydrogen peroxide and helps in its survival in the host.

It has another cell bound material called clumping factor which helps in binding of fibrin and fibrinogen to their cell wall and safeguards them.

The enzyme coagulase exists in free and bound forms which bind to prothrombin then becomes active and converts fibrinogen to fibrin and this fibrin forms a coat around the bacterial cells and make them resistant to opsonisation and phagocytosis.Tube coagulase test has specificity of 98.1% and sensitivity of 98.7%.

Enzyme fibrinolysin which is produced by Staphylococcus acts by breaking the fibrin clots and enhances the spread of infection.

Hyaluronidase hydrolyses the intracellular matrix and aids in the spread of organism in tissues.

Three different types of β-lactamase enzymes are producd by Staphylococcus aureus these enzymes may be inducible(i.e., produced only in the presence β lactum antibiotics) or constitutive(i.e., produced continuously), genes which code for these enzymes are found on plasmids. These resistance genes may be transferred to other bacteria by transformation and transduction.

V) Haemolysins:

Four types of haemolysins are produced by Staphylococcus . α-hemolysin is a heterogenous protein, acts as neurotoxin by causing demyelination of myelin sheath and this hemolysin is responsible for the hemolysis observed around colonies of staphylococcus aureus grown in sheep blood agar .

β hemolysin is a sphingomyelinase which acts on sphingomyelin present in many cells mainly human red blood cells, it is also known as hot-cold hemolysin.

Delta hemolysin is produced by more than 97% of Staphylococcus aureus strains they act as surfactants and damage cell membrane.

γ hemolysin is made of 3 proteins and along with 2 proteins of Panton valentine leukocidin(PVL) form six set of two component toxins, this hemolysin lyses leukocytes efficiently.

Vl) Toxins:

There are three important toxins produced by Staphylococcus aureus and these are Exfoliative toxin, Toxic shock syndrome toxin(TSST), and Entero toxin. Each toxin has got different action on different tissues or systems. Exfoliative toxin cause dermal exfoliation. TSST is responsible for toxic shock syndrome. Enterotoxins are heat stable and are responsible for food poisoning.

Vll)Superantigens.

The Staphylococcal enterotoxins along with toxic shock syndrome toxin are collectively called pyrogenic toxin superantigens.

1) They act on variable domain (Vβ) of T cell receptor
2) Do not require antigen presentation by macrophage
3) Directly stimulate non specific T cells
4) Leads to massive release of cytokines and polyclonal B cell activation .

Identification tests:

l) Morphology in culture medias:

Staphylococcus aureus grows well in simple media.

Nutrient agar: in nutrient agar it appears as smooth, low convex, glistening, densely opaque. The pigment formation in nutrient agar when kept in room temperature in aerobic atmosphere ranges from cream colour to golden yellow due to presence of carotenoids.

ll) Biochemical characters:

Staphylococcus aureus produces catalase, ferments 10% mannitol to acid and 10% trehalose to acid. It liquefies gelatine by enzyme gelatinase, produce deoxyribonuclease(D Nase), produce heat stable thermonuclease(T Nase) when grown in toluidine blue DNA agar producing a pink halo around the well. Indole negative, but urease is produced and gives positive reaction when grown in Christensen's urea medium.

The single most reliable test for identification of Staphylococcus aureus is the coagulase test. There are two types of tests, one is Slide coagulase test that detects bound coagulase(clumping factor) present in S aureus and absent in most other Staphylococcus species. The other one is Tube coagulase test, that detects the presence of free coagulase which reacts with clot retracting factor(CRF) present in plasma, which in turn reacts with fibrinogen to form fibrin.

lll) Rapidec Staph:

It is a rapid kit test which detects the presence of enzyme aurease specific to coagulase positive Staphylococci, the enzyme aurease reacts with prothrombin to form a complex that lyses the substrate to release a flurogen which is detected by its fluorescence under ultraviolet radiation at 365 nm. Staphylococcus is identified within 2 hours with the help of this kit method and also identifies MRSA strains that are not detected by conventional tests.

Klebsiella:

Klebsiella species are usually found as commensals in human intestines and as saprophytes in soil. K.pneumoniae has three sub species: K. Pneumonia subspecies pneumoniae : It is the most pathogenic and common among all and it is responsible for pyogenic infections such as abscesses and wound infections .It frequently colonizes the oropharynx of hospitalized patients and is a common cause of nosocomial infections. Most of the hospital strains are multidrug resistant.

Biochemical properties:

Gram staining: Klebsiella is short, plump, straight gram-negative rods, about 1-2 µm X 0.5-o.8µm in size

Culture: On MacConkey agar, it produces large, dome shaped, mucoid, sticky, pink colour lactose fermenting colonies.

Biochemical identification of Klebsiella:

Klebsiella species can be identified by the following properties.

ICUT test:

Indole test: Negative

Citrate test: Positive(citrate is utilized)

Urease test:Positive(urea is hydrolysed)

TSI(triple sugar iron agar test): Shows acid/acid, with gas formation, H2S absent.

Sugar fermentation test:

Ferments most of the sugars such as glucose, lactose, mannitol, maltose(but not sucrose) with production of acid and gas

VP(Vogas- Proskauer) test: Postive

MR(methyl red) test: Negative

Antigenic Structure

1.Capsular (K) Antigen

On the basis of capsular (K) antigens, the Klebsiellae have been classified into 80(1-80) serotypes. Identification of these capsular antigens is usually done by capsular swelling reaction with specific capsular antiserum.

2.Somatic (O) antigen

Klebsiella contain five (01-05) different somatic or O antigens in various combinations. Four of these (01, 03, 04, 05) are identical or closely related to O antigens of E Coli.

Methods of typing

Phage typing, biotyping, bacteriocin typing(klebocin or pneumocin) and resisto typing have been tried. Many Klebsiella strains produce bacteriocins known as klebocins or pneumocins which show a narrow range of activity on other Klebsiella strains. Klebocin typing can be done by the help of liquid preparations of bacteriocins. Klebocin typing and capsular serotyping together may be very useful for epidemiological studies.

Pseudomonas aeruginosa:

The genus Pseudomonas comprise of aerobic, Gram negative, non-fermentative, non-sporing, oxidase positive bacilli which are motile by polar flagella. Many species produce water soluble pigments which diffuse through the culture medium. Majority of them are saprophytic being found in soil, water, sewage, or wherever decomposing organic matter is found.

1) **Culture**

It is a strict aerobe, and grows well on ordinary media like nutrient broth and nutrient agar. The optimum temperature for growth is 37ºC.

a) **Nutrient agar:** Colonies are smooth, large, translucent, low convex, 2-4 mm in diameter. The organism produces a sweetish aromatic odour. There is greenish blue pigment which diffuses into the medium.

b) **Blood agar**:Colony characters are similar to those on nutrient agar. Many strains are haemolytic on blood agar.

c) **MacConkey agar:** Colonies are pale or colourless(non-lactose fermenters)

d) **Cetrimide agar**: It is a selective medium for Ps. aeruginosa.

e) **Peptone water**: It forms a turbidity with surface pellicle since it is a strict aerobe.

3.Pigment production

Ps.Aeruginosa produces a number of pigments which diffuse into surrounding medium. These pigments are 1)Pyocyanin, 2)Pyoverdin, 3)Pyorubin, 4)Pyomelanin.

4.Biochemical reactions

Ps. Aeruginosa is a non-fermentor, derives energy from carbohydrates by oxidative breakdown. .It utilises only glucose oxidatively with acid production. Lactose and maltose are not utilised. They are catalase, oxidase, and citrate positive. Indole, MR, VP, and H2S tests negative. They reduce nitrates to nitrites.

5)Resistance

It is killed by heating at 55◦C for 1 hr. It is resistant to chemical disinfectants and can even grow in certain antiseptics like quaternary ammonium compounds, chloroxylenol and hexachlorophene. It is intrinsically resistant to commonly used antibiotics. Examples of clinically effective antibiotics are polymyxin B, colistin, piperacillin, tricarcillin, cefotaxime, gentamycin, azlocillin, tobramycin, and ciprofloxin.

6)Antigenic structure

O Antigens: It possesses 19 distinct group specific O antigens, and they are heat stable.

H antigens: Two heat labile H antigens have been recognised in Ps. aeruginosa.

7)Toxins and Enzymes

Several toxins and enzymes produced by Ps. aeruginosa contribute to enhance its virulence

a)Exracellular products: pyocyanin inhibits mitochondrial enzymes in mammalian tissue and causes disruption and cessation of ciliary beat on ciliated nasal epithelium.

b)Extracellular enzymes and Haemolysins: it produces proteases, haemolysins, and lipase. These play a key role in producing local lesions.

c)Exotoxins: it produces two exotoxins A and S. Exotoxin A is a polypeptide. It inhibits protein synthesis, its mechanism of action is similar to that of diphtheria toxin.

d) Endotoxins: it is a lipopolysaccharide(LPS), exhibiting many biological properties of enterobacterial LPS including pyrogenic action.

8)Typing methods

As Ps.aeruginosa ia an important cause for hospital acquired infections, it is essential to type the strain for epidemiological study.

a) Bacteriocin(pyocin) typing: three types of pyocins are produced by Ps. aeruginosa. These pyocins are known as R, F and S. Pyocin – producing strains are resistant to their own pyocins though they may be sensitive to pyocins produced by other strains.
b) Phage typing: considerable difficulties have been encountered in bacteriophage typing.

c) Serotyping: based on O and H antigens, 17 serotypes of Ps. aeruginosa are recognised. It is said to be reliable but facilities exist only in reference laboratories.

d) Molecular method: restriction endonuclease typing with pulsed field gel electrophoresis(PAGE) is the most reliable typing method

Proteus

These are motile, Gram negative bacteria characterised by swarming growth on agar. They were named Proteus because of their pleomorphic character, after the Greek God Proteus who could assume any shape.

Morphology

These are Gram negative bacilli measuring 1-3 x 0.5µm insize. They are non capsulated, non-sporing, and actively motile. They possess peritrichate flagella. They may present as short coccobacillary forms, long and filamentous type especially in young cultures.

Culture

They are aerobic and facultative anaerobic. They grow on ordinary media and culture emits a characteristic putrefactive (fishy or seminal) odour. When grown on nutrient agar or blood agar, it exhibits swarming. Swarming of Proteus appears to be due to vigorous motility of the bacteria.

They form smooth, pale, or colourless(NLF) colonies on MacConkey's agar and do not swarm on this medium. In liquid

medium(peptone water); it produces uniform turbidity with a slight powdery deposit and an ammoniacal odour.

Dienes Phenomenon

When two different strains of Proteus species are inoculated at different places of the same culture plate, swarming of the two strains remain separated by a narrow, visible furrow. However in case of two identical strains of Proteus, swarming of the two coalesce without signs of demarcation. It is known as Dienes phenomenon. It has been used to determine the identity or non-identity of different strains of Proteus.

Biochemical Reactions

The distinctive characters of this genus are: 1) Deamination of phenyl alanine to phenyl pyruvic acid(PPA test). It is always positive.

2)Hydrolysis of the urea by enzyme urease- it is another characteristic of Proteus

3)They ferment glucose by producing acid and gas. Lactose is not fermented. Indole is formed by Pr. vulgaris, but is negative in Pr. mirablis.

4) They are MR- positive, VP-negative

Antigenic structure

The bacilli possess thermostable, 'O'(somatic)and thermolabile 'H'(flagellar) antigens, based upon which several serotypes have been recognised. Weil and Felix noted that certain non motile strains of Pr. vulgaris, called the 'X' strains were agglutinated by sera of typhus patients. The sharing of polysaccharide antigens of Proteus with some Rickettsiae forms the basis of the Weil and Felix reaction for the diagnosis of some rickettsial infections. These non motile strains of Proteus are employed as the antigens for this test-Pr. vulgaris strains OX 2, OX 19, and Pr. mirablis OX K.

Treatment strategies (4,5,7,11)

The ultimate goal of a successful therapy is a long term pain-free and functional joint and limb. Eradication of an orthopaedic device related infection is best achieved by combination of both, an appropriate surgical procedure and a prolonged antimicrobial treatment.

The surgical treatment strategies include:

a)If the duration of symptoms are<3 weeks,stable implants,absence of sinus tract and susceptibility to antibiotics with activity against surface-adhering microorganisms,the the implants can be retained and only debridement can be done.

b)If an implant is not necessary to maintain bony stability, it should be removed.

c) Implants needed for stability should be retained until there is bony stability, or they should be replaced by another form of fixation(e.g., removing a plate and replacing it with an external fixator).

d)If infections are not treated aggressively , surgical fixation can be compromised. It is easier to treat a stable healed fracture with osteomyelitis than an unstable infected non-union fractures.

Infected implants can be removed by

i)The traditional One- stage exchange includes the removal and implantation of a new prosthesis during the same surgical procedure, and has a success rate of 86% to 100% in appropriately selected patients.

ii) The two-stage exchange includes removal of the prosthesis with implantation of a new prosthesis during a later surgical procedure. The two-stage procedure has the highest success rate usually exceeding 90%

iii).Permanent removal of the device is usually reserved for patients with a high risk of reinfection(eg. Severe immunosupression, IV drug users etc) or when no functional improvement after surgery is expected.

In addition, for all cases of ODRI, a long term suppressive oral antimicrobial therapy is the main stay of treatment, Initially I.V antibiotics for 2-6 weeks followed by oral therapy for 3 months in case of internal fixation and hip prosthesis, 6 months for knee prosthesis.

Most of the studies(15) have shown that early post operative infections are associated with more virulent isolates like Staphylococcus

aureus, Klebsiella species and Pseudomonas aeuroginosa, whereas delayed and late post operative infections are associated with low virulent isolates like CONS.

Antibiotic treatment options:

Many a time, prolonged administration of systemic antibiotics may not be effective, hence the concept of local antibiotic delivery system has come up. The advantage is extremely high level of local antibiotic concentration. This facilitates the delivery of antibiotics by diffusion to avascular areas of wound. The resistant organisms start responding to this high concentration. The local antibiotics can be delivered through Non-degradable beads-PMMA(poly methyl metha acrylate) beads/Spacers or Bio-degradable beads like bone graft, bone graft substitutes, natural polymers, synthetic polymers, composite biomaterials etc.[5]

Treatment of Gram Positive organisms:

The main drugs used in the treatment in case of implant infections by gram positive cocci such as staphylococcus aureus are;

i)beta-lactum antibiotics(penicillins, third generation cepahalosporins, monobactums, and carbapenems).

ii)glycopeptides antibiotics(vancomycin)

iii)ribosomal inhibitor like macrolide licosamide, streptogramin B (erythromycin, clindamycin)

iv)aminoglcosides(gentamycin, amikacin)

v)tetracyclines,doxycycline

vi) DNA gyrase blocking drugs(quinolone) and

vii) antimetabolite(trimethoprim) , sulphonamide(sulfamethoxazole)

Antimicrobial Resistance exhibited by Staphylococcus aureus:

A) Resistance to beta-lactum antibiotics-**mecA gene:**

Beta-lactum antibiotics interfere with synthesis of bacterial cell wall by inhibiting transpeptidases so that cross linking in cell wall is inhibited. These enzymes and related proteins are called as penicillin binding proteins (PBPs) located in bacterial cell membrane. Penicillin was the first antibiotic to be used clinically in 1941 and it was the drug of choice for treatment of serious staphylococcus aureus infections. These organisms acquire resistance due to

i)Acquisition of plasmid borne genetic elements coding for beta-lactamases(penicillinase) which opens the beta-lactum ring and inactivates penicillin.

ii) The presence of an altered penicillin binding proteins(PBP).

There are about six different types of PBPs which are mostly transpeptidation enzymes. PBP 2A is mainly responsible for the emergence of MRSA and it is encoded by a gene called mecA. The mecA gene may be expressed by some or all the cells in a given population.

Methicillin resistant staphylococcus aureus gene contains a resistant island called staphylococcus chromosomal cassette(SCC)mec. Here mec is the genetic element that confers resistance to methicillin. Some staphylococcus aureus lack mecA gene but exhibit resistance to

penicillinase resistant penicillins. This type of resistance is due to hyperproduction of betalactamase enzyme which results in slow hydrolysis of semisynthetic penicillin. The gold standard method of detection of mecA gene in MRSA is PCR(polymerised chain reaction).

There are six types(l to Vl) of SCCmec according to structure of their ccrA-ccrB and mecA complexes. After emergence of MRSA strains, glycopeptides antibiotics became the drug of choice in treating these severe infections. But by May 1996, Vancomycin intermediate staphylococcus aureus (VISA) was reported in Japan.

Vancomycin acts by inhibiting cell wall synthesis by binding to D-ala-D-ala terminal and block both transpeptidation and transglycosylation. The VISA strains arises fron chromosomal mutations that affect the structure of the cell wall peptidoglycan. VISA strains have thick cell wall that contains increased number of free uncrosslinked D-ala-D-ala terminals and these act as traps before glycopeptide molecules reach their target.

The CLSI(Clinical laboratories standards institute) defines staphylococcus requiring Vancomycin concentration of < 2µg/ml for growth inhibition as susceptible those requiring 4 to 8 µm/ml as intermediet and those requiring ≥16µm/ml as resistant

B) Resistance to Macrolide-Lincosamide-Streptogramins-B(MLS) group of drugs-**erm Gene: (16)**

Many staphylococcus aureus strains have developed resistance to macrolide-lincosamide- streptogramins(MLSb) which are due to:

- Modification of target site
- Active efflux of the drug
- Inactivation of the drug

The organism which are resistant to Erythromicin are almost resistant to other MLS group of drugs.These antibiotic molecules bind to bacterial ribosomes and block protein synthesis. Ribosome modification is mediated by erm gene which codes for erythromycin methylase that decreases the affinity of the drug to its target. These erm determinants are located in mobile genetic elements such as transposons or plasmids.

Resistant Staphylococcus aureus to MLS group of drugs could be due to the expression of erm gene and these strains could be,

i)constitutively resistant strains that can grow in the presence of a high concentration (more than 100 μg/ml) of MLS antibiotics without prior induction and

ii) inducibly resistant strains whose high-level resistance to MLS antibiotics can be induced by subinhibitory concentrations (0.01 to 0.1 μg/ml) of Erythromicin.

Among MLSb drugs , macrolides are good erm inducers. Once if they are induced the gene product confers cross resistance to other members of MLSb group of drugs, this type is called inducible resistance(i.e.,diffusion of erythromycin towards clindamycin disk induces clindamycin resistance as a result the zone of inhibition around clindamycin takes D-shape).But in

some strains the erm gene undergoes mutation and results in constitutive resistance expression(i.e.,inhibition zone around clindamycin).

Treatment of Gram Negative organisms:

i)Aminoglycosides like Amikacin,Gentamicin

ii)DNA gyrase inhibitors like Ciprofloxacin, Ofloxacin.

iii) 3rd and 4th generation Cephalosporins like Cefotaxime, Ceftrioxazone, Ceftazidime

iv)Anti pseudomonal penicillins like Piparacillin

v)beta-lactamase inhibitor like Tazobactum

Antibiotic resistance by Gram negative organisms:

ESBL(extended specrum beta lactamases) : Increased use of antibiotics, particularly third generation of cephalosporins, has been associated with the emergence of β-lactamases mediated bacterial resistance, which subsequently lead to the development of ESBL producing bacteria. ESBL are the enzymes that mediate resistance to extended spectrum e.g., third generation cephalosporins as well as monobactams such as Aztreonam(CLSI,2010). These enzymes catalyze the hydrolysis of the β-lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBL have been reported worldwide in many different genera of enterobactericeae and pseudomonas aeroginosa. However these are the most common in Klebsiella pneumoniae & E Coli. Few unique characteristics of ESBL are as follows:

a) Mostly class A cephalosporinases and are plasmid mediated.
b) More common in Klebsiella species followed by E Coli
c) All enzymes are active against Cephalothin.
d) Imipenam and cefoxitin are not hydrolysed.
e) Comparative activity against cefotaxime and ceftazidime varies with the enzyme
f) Inhibition activity by β –lactamase inhibitors can be demonstrated.

The predisposing factors for ESBL production are prolonged stay in intensive care units, long term use of antibiotics, very severe illness, extensive use of third generation cephalosporins, use of life lines and catheters.

TEM type of ESBLs are the derivatives of TEM-l and TEM-2. TEM-1 was first discovered in a patient called Temonieria and named as TEM. More than 100 types of TEM ESBLs have been described. TEM-1, 2, 13 are not ESBLs.

SHV was named so because they are sulfhydryl variable. They are the most commonly found type of ESBL in the clinical isolates. It was first isolated from Klebsiella ozaenae in 1983. More than 50 types have been reported so far.

CTX-M are ESBLs which have tendency to hydrolyse Cefotaxime more than Ceftazidime and also hydrolyse Cefepime with more efficiency. More than 113 types have been described.

OXA-β lactamases have the ability to hydrolyse Oxacillin and Cloxacillin. The other types of ESBL includes PER1-2, VEB1-2, GES, SFO, IBC etc.

Recent reports describe ESBLs a global threat to health(Pitout and Laupland,2008) and the Infectious Disease Society of America has listed the ESBL producing Enterobacteriaceae as one of the six problematic drug-resistant pathogens and reported the urgent need for newer and more effective therapeutics.

Prevention of ODRI infection

1) **Preoperative methods**
   - ❖ Patient-specific factor optimisation
   - ❖ MRSA decolonization
   - ❖ Skin disinfection
2) **Intraoperative methods**
   - ❖ Antibiotic prophylaxis
   - ❖ Cutaneous preparation(hair removal, skin antisepsis, and surgical draping)
   - ❖ Operative environment(operating theatre ventilation, body exhaust suits, gloves, and intraoperative lavage)
   - ❖ Blood conservation
   - ❖ Prosthesis selection
3) **Postoperative methods:**
   - ❖ Antibiotic prophylaxis

- ❖ Evacuation drains

Antimicrobial prophylaxis:

The aim of antibiotic prophylaxis is to ensure effective serum and tissue levels of drugs during surgery. Certain guidelines were recommended to start antimicrobial prophylaxis before surgery, they are

- Prophylactic antibiotics should be initiated within 1 hour before incision
- Antibiotics should be administered in accordance with surgical procedure
- Prophylactic antibiotic should be discontinued within 24 hours of surgery except cardiothoracic surgery (discontinued within 48 hours)

A multicentric study conducted in united states proved that of antibiotics administered within 1 hour before surgery reduce infection rate and this was reduced further if administered within 30 minutes before incision.

Single infusion of antibiotics given 1 hour before incision gives sufficient protection and it has to be repeated intraoperatively for procedures lasting more than 24hours and when substantial blood loss occurs. Antibiotics must be present in the surgical site throughout the surgical period. There is no benefit in continuing antibiotic prophylaxis after 24 hours of surgery except cardiac surgery.

Treatment of orthopaedic device related infections is a scenario where many variables play a role in deciding the treatment. Therefore, the best treatment strategy is prevention of infection. Antimicrobial prophylaxis remains the most effect method of reducing the prevalence of infection in fracture fixation surgeries.

In orthopaedic surgery, a first-generation or second generation cephalosporin , such as cefazoline or cefuroxime, is a rational choice. If the patient is allergic to cephalosporins or in settings with high prevalence of methicillin resistant S. aureus(MRSA), vancomycin or teicoplanin are alternative options.

For optimal efficacy of the prophylactic agent , antimicrobial inhibitory concentrations must be achieved in tissues at the time of incision and last during the entire procedure. When a tourniquet is used, tissue concentrations of the antibiotic are usually insufficient for prevention of implant infection when administer 5 min before inflation or later.

For internal fixation devices of closed fractures in centres with infection rates of less than 5%, a single dose of intravenous cefuroxime is a reasonable option. In centers within known or high infection rates(> 5%) and in open fractures grade l and grade ll , a 1- day prophylaxis is preferred : intravenous cefuroxime and /or tazobactum-pipracillin may be used. In patients with internal fixation of grade lll open fractures, preventive therapy with an anti staphylococcal drug such as intravenous amoxicillin/clavulanic acid or cefuroxime for 5-7days is reasonable.

Haematogenous seeding of implants may occur during the whole life, although the risk of infection is highest in the first year after implantation. The most frequent sources of haematogenous infection are infections of skin and soft tissues, oral cavity, urinary and respiratory tract. They must be tackled accordingly.

MATERIALS & METHODS

MATERIALS AND METHODS

Place of Study

This study on “Isolation and characterisation of bacterial pathogens in orthopaedic implant associated infections” was conducted in the Department of Microbiology, Coimbatore Medical College Hospital, Coimbatore.

Study design

A hospital based Prospective Study

Duration of study

The study was conducted for a period of one year from July2016 to June2017

Approval

Ethical clearance was obtained from Institutional Ethics Committee prior to the conduct of the study and informed consent was obtained from all patients included in the study.

Sample size

This study was carried out in 136 cases with implant infections from patients admitted in orthopaedic and plastic surgery wards.

Data Analysis

Data entry was made in the Excel Software and Analysis was done with SPSS-24 computer package. The categorical variable is expressed in frequency and percentage. The continuous variable is expressed in terms of mean and standard deviation. The associations between variables was found by chi-square test and independent sample t-test P value <0.05 was considered statistically significant.

Inclusion criteria

1) Patients with implant infections in orthopaedic and plastic surgery wards
2) Patients of all age groups and both sexes with implant infections.
3) Patients with open/closed fracture.
4) Surgeries including emergency and elective procedures.

Exclusion criteria

1) Implantation done through already infected wound.
2) Patients on long term steroids and immunosuppressive therapy.
3) Patients with chronic illness like Tuberculosis, Malignancy.
4) Patient with concurrent urinary tract infection.

Evaluation

Data collection include name, age, address, date of admission, diagnosis at admission, physical examination finding, duration of hospital stay, nutritional status, type of implant, duration of procedure, any underlying

illness(urinary tract infection, respiratory tract infection),co morbid conditions(diabetes mellitus, tuberculosis, malignancy),drug history(steroids, immunosuppressivedrugs),smoking and alcoholism were also recorded**.**

Sample collection and transportation

The pus samples were collected aseptically from patients presented with clinical evidence of infection (purulent discharge from incision site or drain or implant) using sterile cotton swabs from surgical site after cleaning the surrounding area with sterile normal saline without touching the wound to prevent contamination by skin commensals. In patients with deep seated abscesses, using sterile syringe under aseptic precaution pus was aspirated and transported to the laboratory immediately for further processing.

Anaerobic cultures were done in cases of suspected anaerobic infections. Specimens of infected tissue bits and periprosthetic membrane were collected during wound debridement in a sterile container containing thioglycollate broth. Gaspak method was used to maintain oxygen free environment during processing of specimen.

Processing of specimens

After proper sample collection under aseptic precaution the same were processed as follows,

- Direct microscopic examination of Gram stained smear

- Inoculation of samples into Nutrient agar, Blood agar, MacConkey agar, and selective media such as Mannitol salt agar
- Thioglycollate broth was inoculated for the isolation of anaerobic organisms
- Preliminary identification of growth by colony morphology
- Biochemical tests for characterisation of species
- Antibiotic sensitivity test

Direct Gram's Stain

For microscopic examination of pus cells and bacteria, the smear was prepared by rolling the pus culture swab on to the surface of a clean, dry sterile glass slide. The smear was allowed to air dry and then gently heat fixed.

Smear was stained by Gram staining method. Once stained the smear was examined under low power microscope for the presence of pus cell, debris, and fungi. Next the smear was evaluated under oil immersion method in 100x objective for the presence of bacteria as well their Gram reaction, morphology and arrangements of the cells.

Culture for Aerobic organisms:

The pus culture swab was inoculated on to Nutrient agar plate, 5% sheep blood agar, MacConkey agar plate and by using sterile bacteriological loop streak culture done and plates were incubated at 37ºC for 24-48 hours. The blood agar plate was incubated at 5-10% CO2

Culture for Anaerobic organisms:

Specimen(aspirated pus or infected tissue bits or periprosthetic membrane)from thioglycollate broth was plated on anaerobic blood agar plates by streak culture method. The plates were placed in the anaerobic gaspak system with a chemical and a biological indicator(pseudomonas aeruginosa) and incubated at 37ºC for 24 hours.

After overnight incubation, culture plates were observed for growth. If there was no growth, plates were further incubated for 48 hours and reported as no growth. The plates which were kept in the anaerobic gaspak system, were observed daily for any growth for minimum of seven days before reported as no growth. If growth was present identification of organisms were done by observing their colony morphology, culture characteristics, Gram staining and biochemical reactions.

Biochemical tests:

- Catalase
- Modified oxidase
- Mannitol salt agar test
- Urease test
- Slide and Tube coagulase test
- Bile esculin test

In case of Gram negative isolates, hanging drop for motility, Catalase, Oxidase, Triple sugar iron(TSI), Nitrate reduction test, Lysine,Arginine,Ornithine(LAO)test, Oxidation and fermentation test(Hugh & Leifson) were also done.

Catalase test

This test demonstrates that presence of enzyme catalase which is present in most of the cytochrome containing aerobic and facultative anaerobic bacteria. Catalase is capable of converting hydrogen peroxide(one of the oxidative end product of aerobic carbohydrate metabolism) to water and oxygen as accumulation of this is lethal to the bacteria.

Using a sterile glass rod, a small amount of colony is transferred to glass tube containing 3% hydrogen peroxide. The rapid and sustained appearance of effervescence indicates positive test

Oxidase test

This test is done to determine the presence of bacterial cytochrome oxidase. Oxidation of the substrate tetramethyl –p-phenylene diamine dihydrochloride to indophenols which is a dark purple colored end product, indicates a positive test.

A small amount of colony was streaked on to the moistened filter paper disks, impregnated with freshly prepared 1% tetramethyl paraphenylene

diamine dihydro chloride. An intense deep blue colour appearing within 5-10 seconds was taken as positive reaction.

Mannitol fermentation test

Colonies of Staphylococcus aureus was streaked on to mannitol salt agar (1% mannitol, 7.5% sodium chloride, phenol red and peptones) and incubated for 24-48 hours at 37ºC. High salt concentration of medium allows the growth of Staphylococci and inhibit the growth of other organisms(except enterococci)

Interpretation:

Yellow zone around colonies indicating acid production from mannitol

Urease test

This test is used to determine the ability of the organism to produce the enzyme urease which hydrolyzes the urea to ammonia which results in alkaline pH & change of colour.

The surface of the agar is streaked with the test organism and incubated at 35ºC for 18 to 24 hours. The appearance of pink colour indicates the production of enzyme urease.

Coagulase test

This test is done to differentiate Staphylococcus aureus from coagulase negative staphylococci. It can be done by slide and tube method.

Slide coagulase test

This test detects bound coagulase. A colony suspected to be Staphylococcus species was emulsified with sterile saline on a clean glass slide to form a milky suspension. A drop of citrated human plasma was added to the suspension. A similar suspension was made with known staphylococcus strains to test the proper reactivity of plasma. Presence of coarse clumping of cocci within 10 seconds indicates that organism was slide coagulase positive. It was confirmed by tube coagulase test.

Tube coagulase test

This test detects free coagulase. Few colonies from blood agar plates were mixed with 0.5ml of diluted plasma in the test tube. Positive control, Negative control, and a tube of undiluted plasma were also kept ready. Tubes were incubated at 35ºC for 24 hours. They were examined at 1, 2, and 4 hours for clot formation. The plasma was converted into a stiff gel that remained in place when the tube was tilted. If no clot was seen, the tube was re-incubated at room temperature and it was read again after 18 hours. Clot formation confirmed the slide test and the organism was identified as Staphylococcus aureus.

Detection of Enterococci:

Bile esculin test

Few colonies from 18-24 hours pure culture was inoculated on to the surface of bile esculin agar slant. Ferric ammonium citrate was used as an indicator and incubated for 24-48 hours at 35ºC

Interpretation:

Positive: Diffuse blackening of more than half of agar slant.

Negative: No blackening of medium was seen.

PYR test(L-pyrro glutamyl aminopeptidase test)

Streptococcus pyogenes and Enterococci isolates were subjected to PYR test. The bacterial colony inoculated into L-pyrrolidonyl-β-Napthylamide agar and incubated at 35-37ºC for 18-24 hours. Presence of the enzyme L-pyrrolidonyl arylamidase in Streptococcus pyogenus or Enterococcus fecalis hydrolyses the substrate and forms β-Napthylamine.

The colonies are picked up with a sterile cotton swab and a drop of PYR reagent (N-N-dimethl cinnamaldehyde) was added to the swab. Developement of red colour(positive reaction) within 5-10 minutes was observed.

Sugar fermentation test

This test is to determine the ability of organism to ferment carbohydrates. A single colony or a drop of liquid culture was inoculated into 5ml of peptone water in a test tube containing 1% sugars(glucose, lactose,

sucrose, maltose, mannitol etc) with a Durham's tube in it and Bromothymol blue as indicator and incubated at 35ºC for 24-48 hours.

Interpretation:

Acid production: Blue coloured medium turns yellow due to acid production

Gas production: Presence of gas bubbles in Durham's tube.

Citrate utilization test:

A well isolated colony was picked up from the he slant surface of MacConkey agar plate and inoculated on to the slant surface of Simmon's citrate agar medium and incubated at 35ºC for 24 to 48 hours. Colour change of medium from green to deep blue with visible colony growth along the streak line was interpreted as positive

Triple sugar iron agar test(TSI)

TSI is used to determine whether Gram negative bacilli ferments glucose, lactose or sucrose and forms hydrogen sulphide(H2S).

Touch the top of a well isolated colony with the help of straight inoculation needle and inoculate into TSI media by first stabbing through the centre of the medium up to half of the butt and then streaking the slant surface. Incubate the tube at 35ºC in ambient air for 18 to 24 hours. The change in colour of the slant/butt with or without gas and black precipitate is noted.

Nitrate reduction test

This is done to determine the ability of the organism to reduce nitrate to nitrite or free nitrogen gas. Test organism was inoculated onto the nitrate reduction broth which contains large amount of nitrate (KNO3). After incubation at 35ºC for 48 hours, alpha napthylamine and sulfanilic acid are added. If nitrate reducing bacteria is present, it will reduce nitrate to nitrite which turns the broth into red colour on adding these two compounds.

Decorboxylase test(Lysine, arginine, ornithine)Moeller's method

This test is used to differentiate decorboxylase producing Enterobactericeae from other Gram negative bacilli. This test is done to determine the ability of an organism to decorboxylate an amino acid to form an amine with resulting alkalinity.

Test organism is inoculated into one tube containing base(Moeller decorboxylase medium) with amino acid to be tested and other tube containing only the base which act as a control tube. Overlay both the tubes with sterile mineral oil for about 1cm of the surface and incubate at 35ºC for 18-24 hours.

The appearance of yellow colour in the control tube indicates that the organism is viable and pH of the medium is sufficiently lowered to activate the decorboxylase enzymes. Reversion of the tubes containing amino acid to a blue purple colour indicates a positive test.

Oxidation fermentation test(Hugh & Leifson)

Principle: To determine the oxidative or fermentative metabolism of a carbohydrate

Fermentation is an anaerobic process and bacterial fermenters of carbohydrates are usually facultative anaerobes. Oxidation is an aerobic process and bacterial oxidizers are usually strict aerobes.

It employs a semi-solid medium in tubes containing the carbohydrate under test and a pH indicator. Two tubes are inoculated and one is immediately overlayed with sterile paraffin oil and sealed to produce anaerobic conditions. Oxidizing organisms produce an acid reaction in the open tube only. Fermenting organisms produce an acid reaction throughout the medium in both tubes. Organisms that cannot breakdown the carbohydrate aerobically or anaerobically produce an alkaline reaction in the open tube and no change in the covered tube.

Antimicrobial susceptibility test

Antimicrobial sensitivity test for all isolates were performed by Kirby-Bauer disc diffusion method on Mueller Hinton agar plate as per CLSI guidelines. Antibiotic discs were procured from Himedia Labs Mumbai.

Inoculum preparation

Using sterile wire loop, 3-4 well isolated colonies of the organism was taken and inoculated into 2-3 ml of peptone broth. Incubate at 37ºC for 2-6 hours to attain a turbidity which corresponds to 0.5 McFarland standard.

Preparation of 0.5 McFarland standard

It is prepared by mixing 1% sulphuric acid and 1.175% barium chloride to obtain a solution with a specific optical density which is comparable to the density of bacterial suspension of 1.5x10 CFU/ml.

Inoculation of test plates

After adjusting the turbidity of suspension, a sterile cotton swab was dipped into the adjusted suspension. The excess inoculums was removed by pressing the swab firmly on the inside wall of the tube above the fluid level.

To the dried surface of Mueller-Hinton agar, the swab was streaked on the entire surface of the agar plate. The plates were dried for few minutes with lid closed. Commercially available antibiotic disks obtained from Hi-Media laboratories Limited were used. Using a sterile forceps the antibiotic disks were placed (6 disks for one 90mm plate) on the inoculated plates and gently pressed to ensure even contact and incubated at 37ºC.

After 16-18 hours of incubation the diameter of each zone size measured using Antibiotic Zonescale and recorded in mm. Once zone size have been measured interpretive criteria are assigned based on CLSI-M2 series“

Performance Standards for Antimicrobial Disk Susceptibility Tests"(M100-S24Suppliments)

Antibiotic discs used for Gram positive isolates were Cotrimoxazole (25µg),Penicillin10units,Gentamicin(15µg),Amoxicillin(30µg),Erythromycin(15µg), Azithromycin(15µg), Clindamycin(2µg), Cephalexin(30µg), Cefoxitin(30µg), Doxycycline(30µg), Ciprofloxacin(5µg) Linezolid(15µg), and Vancomycin.

Antibiotic discs used for Gram negative isolates were Gentamycin(30µg),Amikacin(30µg),Ciprofloxacin(5µg),Cefazoline(30µm),Ceftrioxone(30µg),Ceftazidime(30µg),Cefotaxime(30µg),Cefipime(30µg),Ampicillin-Sulbactum(10µg),Meropenem(10µg), and Pipracillin-Tazobactum (100µg) were used.

Phenotypic screening test for Staphylococcus aureus

The Staphylococcus aureus isolate was inoculated in nutrient broth and incubated till the inoculum reaches 0.5 McFarland turbidity, and following phenotypic screening tests were performed.

Screening test for mec-A mediated Oxacillin resistance

A lawn culture was done in MHA plate as for standard disc diffusion by Kirby-Bauer method. Cefoxitin 30µg disc was placed and incubated at 35ºC in ambient air for 24 hours. The AST plate was examined in reflected light, a

zone of inhibition below 21mm is taken as mec-A positive(MRSA) and zone diameter more than 22mm is taken as mec-A negative (MSSA) strain.

Methicillin resistance is mediated by acquired penicillin binding protein 2A (PBP2A), encoded by chromosomal gene called mec-A.PBP2A has special requirement for peculiar cell wall precursors. The strains positive by this test were labelled as Methicillin Resistant Staphylococcus aureus (MRSA). Cefoxitin is used as a surrogate marker for MRSA.

Inducible Clindamycin resistance D-test

A lawn culture of Staphylococcus aureus isolate was done in MHA plate. Macrolide antibiotic Erythromycin 15µm disc was placed and Lincosamide antibiotic Clindamycin 2µm disc placed 15-25 mm apart. The plate was incubated in 35-37ºC in ambient air for 16-18 hours.

Flattening of zone of inhibition around clindamycin adjacent to the erythromycin disc resulting in D-shape was considered positive. The inducible resistance is due to erm B gene of Staphylococcus aureus and macrolides are potent erm B inducers. If there is no zone of inhibition around clindamycin it is taken as constitutive resistance.

Screening of Vancomycin Minimal Inhibitory Concentration (MIC) by E test

Vancomycin disc diffusion does not differentiate between Vancomycin susceptible isolates from Vancomycin intermediate isolates. The high molecular weight antibiotics like Vancomycin do not diffuses according to concentration gradient, while diffusing through the MHA medium.

MIC is the lowest concentration of antimicrobial agent that inhibits visible growth. Commercial method of MIC determination is done by E test (Himedia Ezy MIC strip Van 0.16-256 mcg/ml).

A lawn culture of the inoculum was made in MHA plate and E strip was brought to room temperature. By using the adhesive applicator stick the E strip placed in the centre of the plate and the applicator stick removed gently. E strip will be adsorbed and will firmly adhere to the agar surface immediately. The MHA plate was then incubated at 35ºC for 24 hours. MIC is determined by examining the point of intersection of bacterial growth with graded concentration of Vancomycin in the E strip which has MICs in the range of 0.016 mcg/ml to 256 mcg/ml.

The interpretive criteria for identifying Vancomycin sensitive, intermediate, and resistant Staphylococcus aureus by E test method are as follows:

- Sensitive if MIC less than 2 mcg/ml.

- MIC in between 4-8 mcg/ml is considered as intermediate resistant (VISA).
- MIC above 16mcg/ml by E test is considered as Vancomycin resistant Staphylococcus aureus (VRSA)

Laboratory methods for identification of Extended Spectrum Beta Lactamases(ESBL)

Gram negative bacteria which are resistant to third generation cephalosporins (Cefotaxime, Ceftriaxone, Ceftazidime, Cefpodoxime) are screened for ESBL production.

ESBL Screening test

The current Clinical laboratory standard Institute(CLSI) guidelines for detection of ESBL includes initial screening test with any four of the following betalactum antibiotics: Cefpodoxime, Cefotaxime, Ceftriaxone, Ceftazidime, Aztreonam. The use of more than one antimicrobial agent for screening improves the sensitivity of detection. Cefpodoxime and Ceftazidime shows the highest sensitivity for ESBL detection. In the present study screening was done by using Cefpodoxime, Cefotaxime,Ceftriaxone, and Ceftazidime.

A lawn culture of the test inoculums was made in Mueller-Hinton agar plate and antimicrobial discs (Cefpodoxime10µg, Cefotaxime 30µg, Ceftriaxone 30µg, Ceftazidime 30µg) were placed and incubated at 35-37ºC for 16-18 hours. After incubation the AST plate was examined and zone size

measured. Zone size of (Cefpodoxime ≤ 17, Cefotaxime ≤ 27, Ceftazidime ≤ 22, Ceftriaxone ≤ 25) indicates ESBL production.

ESBL confirmatory tests

Phenotypic confirmatory testing depends on demonstrating a synergy between Clavulanic acid and an indicator Cephalosporin. Many variations of confirmatory testing have been described but few are convenient for routine use: combined disc method, double disc(DD)approximation test, minimum inhibitory concentration (MIC) methods, and E –test ESBL strips. The CLSI recommends the combined disc method, and the MIC method for ESBL confirmation. In the present study combined disk test was used for phenotypic confirmation.

Combined disk test

It depends on comparing the inhibition zones around disks containing an indicator Cephalosporin with and without Clavulanic acid. As per CLSI guidelines, 10µg of Clavulanic acid is added to each of a Cefotaxime (30µg) and a Ceftazidime (30µg) disk. If ESBL is produced, the zone diameters given by the disks with Clavulanic acid are > 5mm larger than those without the inhibitor. ESBL production is inferred if the zones given by the disks with clavulanate > 5mm larger than those without the inhibitor.

Molecular Identification of Antimicrobial Resistance Gene:

Materials & Methods:

Purefast Bacterial DNA minispin purification kit [Kit contains Lysozyme, Lysozyme digestion buffer, Proteinase-K, Binding buffer, Wash buffer-1, Wash buffer-2, Spin columns with collection tube and Elution buffer]. HELINI 2X RedDye PCR Master mix, Agarose gel electrophoresis consumables and MecA Primers are from HELINI Biomolecules, Chennai, India.

2X Master Mix:

It contains 2U of Taq DNA Polymerase, 10X Taq reaction buffer, 2mM MgCL2, 1µl of 10mM dNTPs mix and RedDye PCR additives.

Agarose gel electrophoresis:

Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide are from HELINI Biomolecules, Chennai.

PCR:

HELINI Ready to use MecA gene Primer mix-5µl/reaction

PCR Product size: 200bp

Bacterial DNA Purification done as per the Purefast Bacterial DNA minispin purification kit protocol.

PCR Procedure:

1. Reactions set up as follows;

HELINI RedDye PCR Master mix- 10µl

HELINI Ready to use gene primer mix- 5µl

Purified Bacterial DNA- 5µl

Total volume- 20µl

2. Mixed gently and spin down briefly
3. Place into PCR machine and programmed it as follows

Initial Denaturation: 94°C for 5min

Denaturation: 94°C for 30 sec }

Annealing: 58°C for 30 sec } 35 cycles

Extension: 72°C for 30 sec }

Final Extension: 72°C for 5min

Loading:

1. Prepared 2% Agarose gel.(2gm of agarose in 100 ml of 1X TAE buffer)
2. Run electrophoresis at 50 V till the dye reaches three fourth distances and observe the bands in UV transilluminator.

Agarose gel electrophoresis:

The PCR Products were analysed by Gel electrophoresis with 2% agarose in 1X TAE buffer with Ethidium bromide (5µl/ml) and visualised by UV- transillumination. A 100 base pair (bp) DNA ladder (HELINI, Chennai) was used as a marker. Duplex- PCR yielded products with size of 200 bp which corresponds to MecA gene.

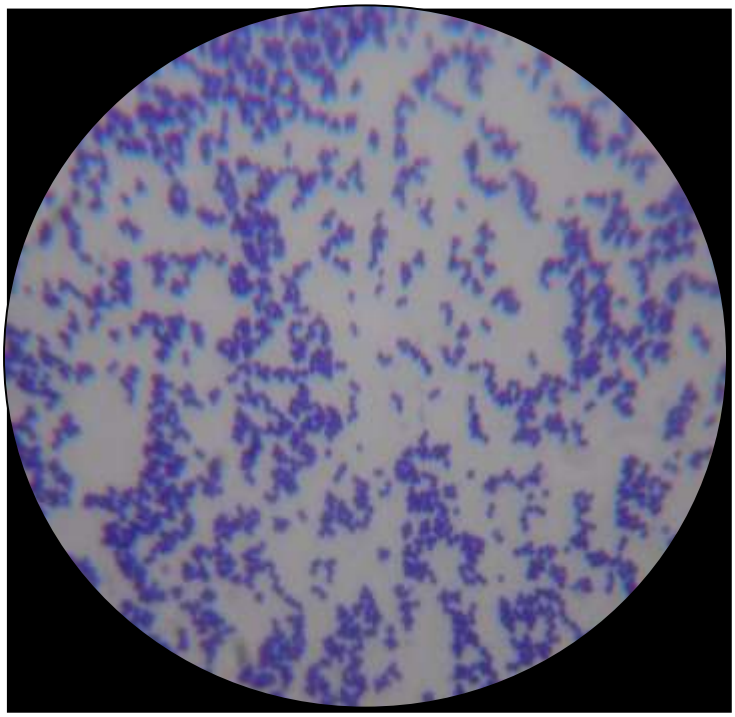


Figure 1: Gram Stain - Gram positive cocci in clusters of Staphylococcus aureus.

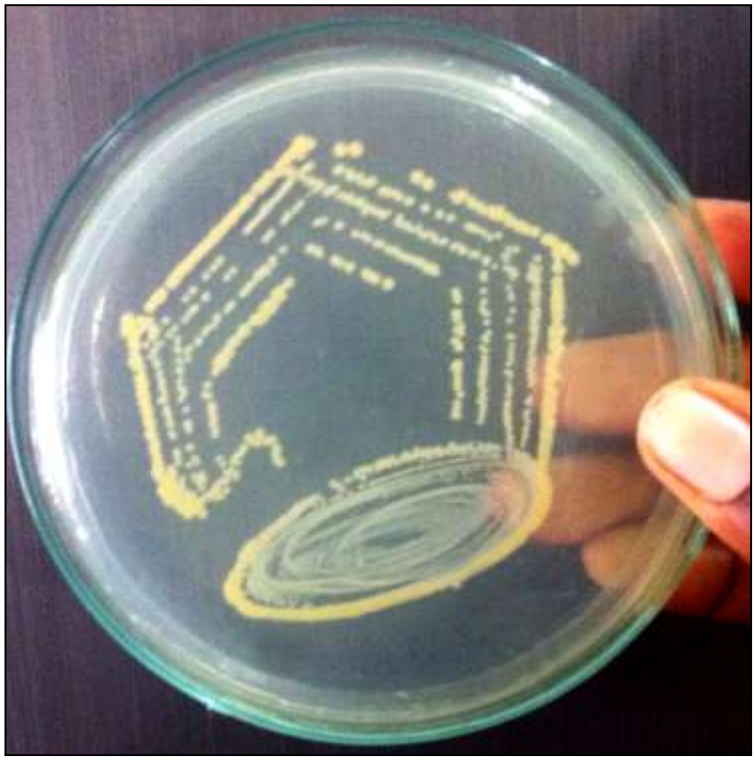


Figure 2: Nutrient Agar – Golden yellow colonies of Staphylococcus aureus.

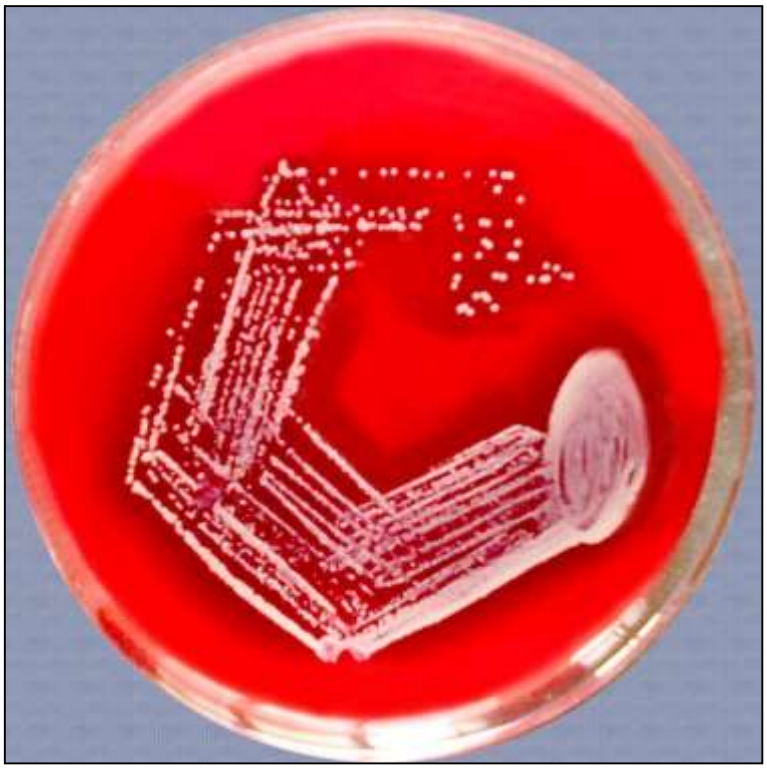


Figure 3: Beta hemolytic colonies of Staphylococcus aureus in 5% sheep blood agar.

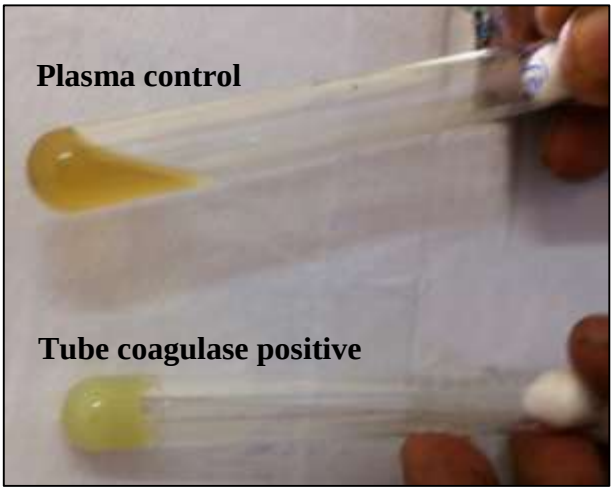


Figure 4a: Tube coagulase test

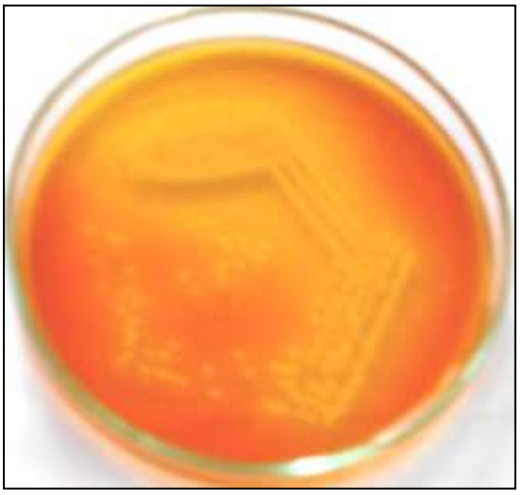


Figure 4b: Mannitol Salt Agar– Yellow colonies of Staphylococcus aureus.

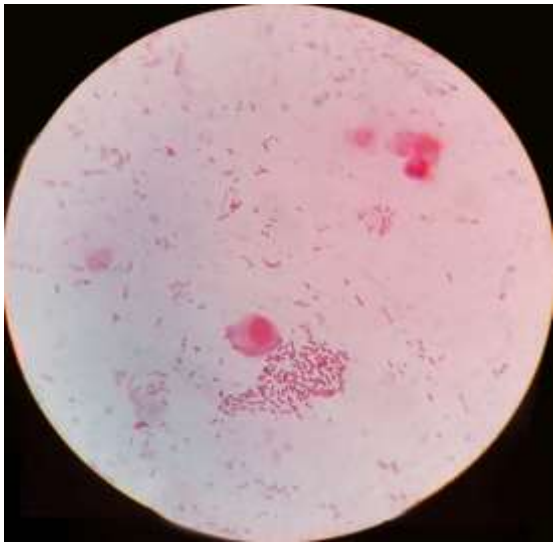


Figure 5a: Direct Gram stain showing pus cells and Gram negative bacilli

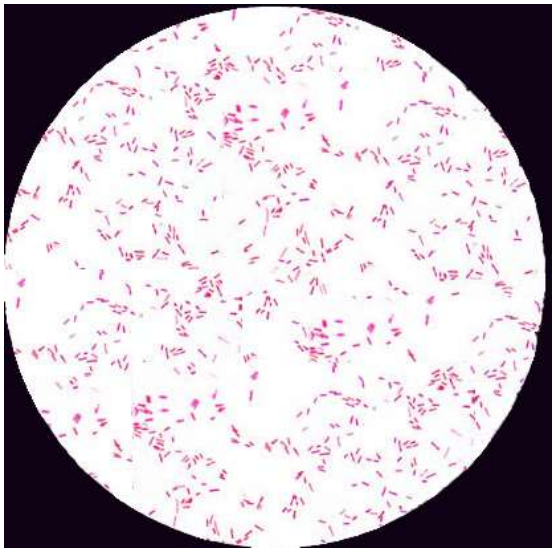


Figure 5b: Gram stain showing Gram negative bacilli

Figure 6: MacConkey Agar: Mucoid Lactose Fermenting Colonies of Klebsiella

Figure 7: MacConkey Agar – Flat lactose fermenting colonies of E.coli

Figure 8: Nutrient agar –Pigmentation of Pseudomonas aeruginosa

Figure 9: Blood Agar Plate - Swarming growth of Proteus

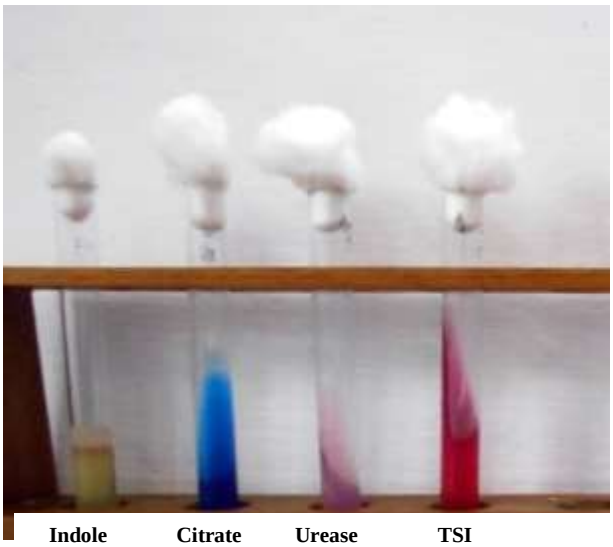


Figure 10: Biochemical reactions of Pseudomonas aeruginosa

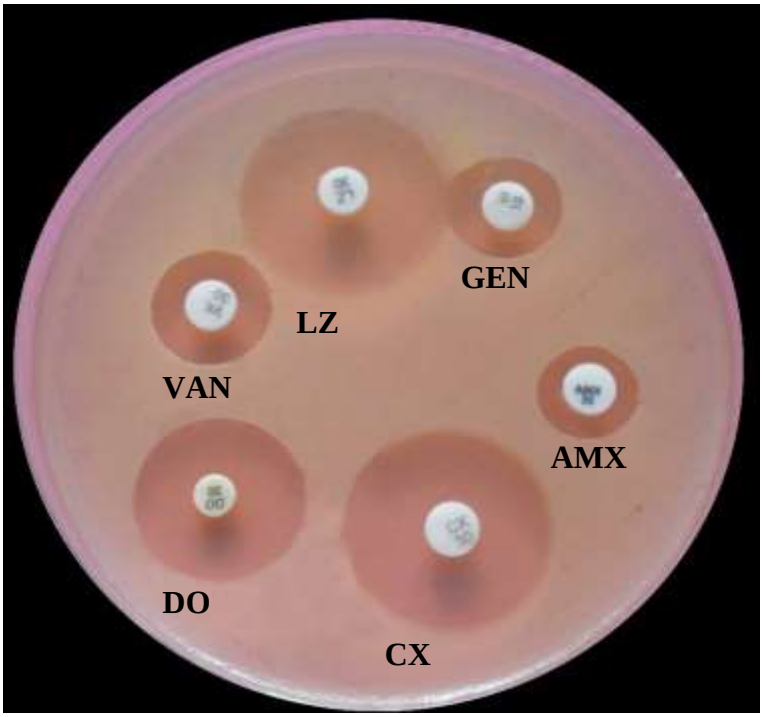


Figure 11: Antibiotic Sensitivity of Staphylococcus aureus by disc diffusion method

Figure 12: E Strip showing Vancomycin sensitivity of less than 2µg/ml for Staphylococcus aureus

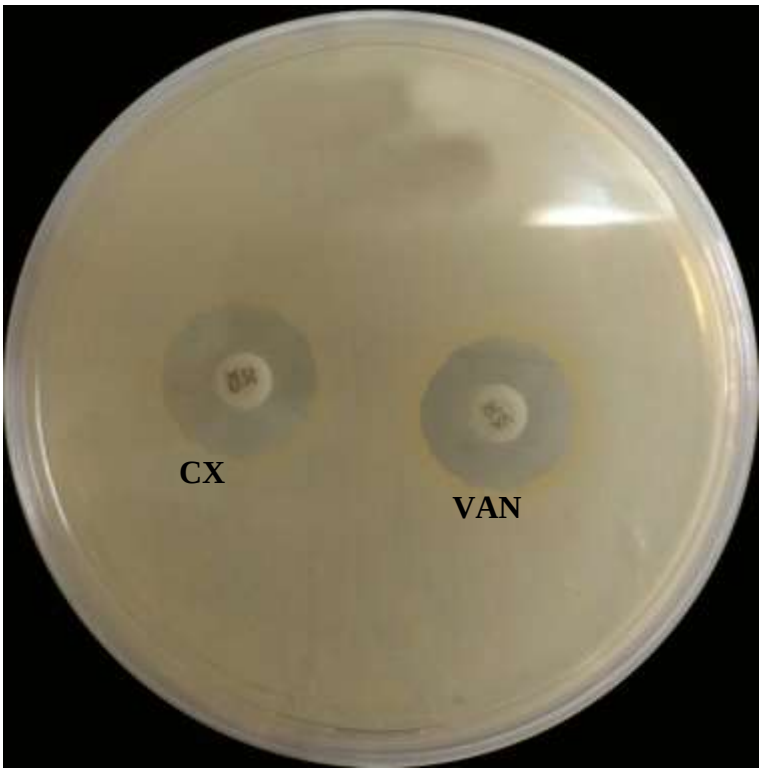


Figure 13: Antibiotic susceptibility of S.aureus resistant to Cefoxitin (MRSA)

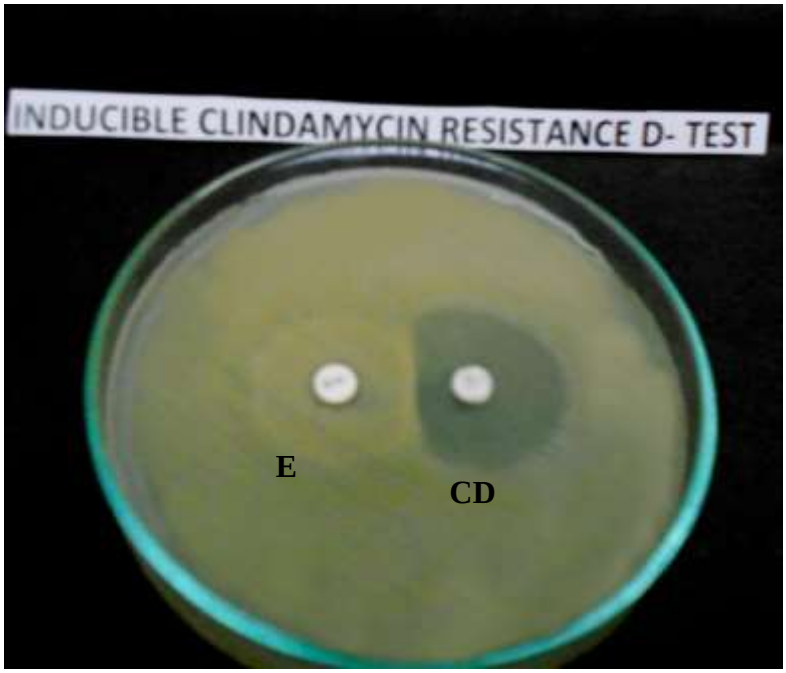


Figure 14: Inducible Clindamycin resistance D-Test

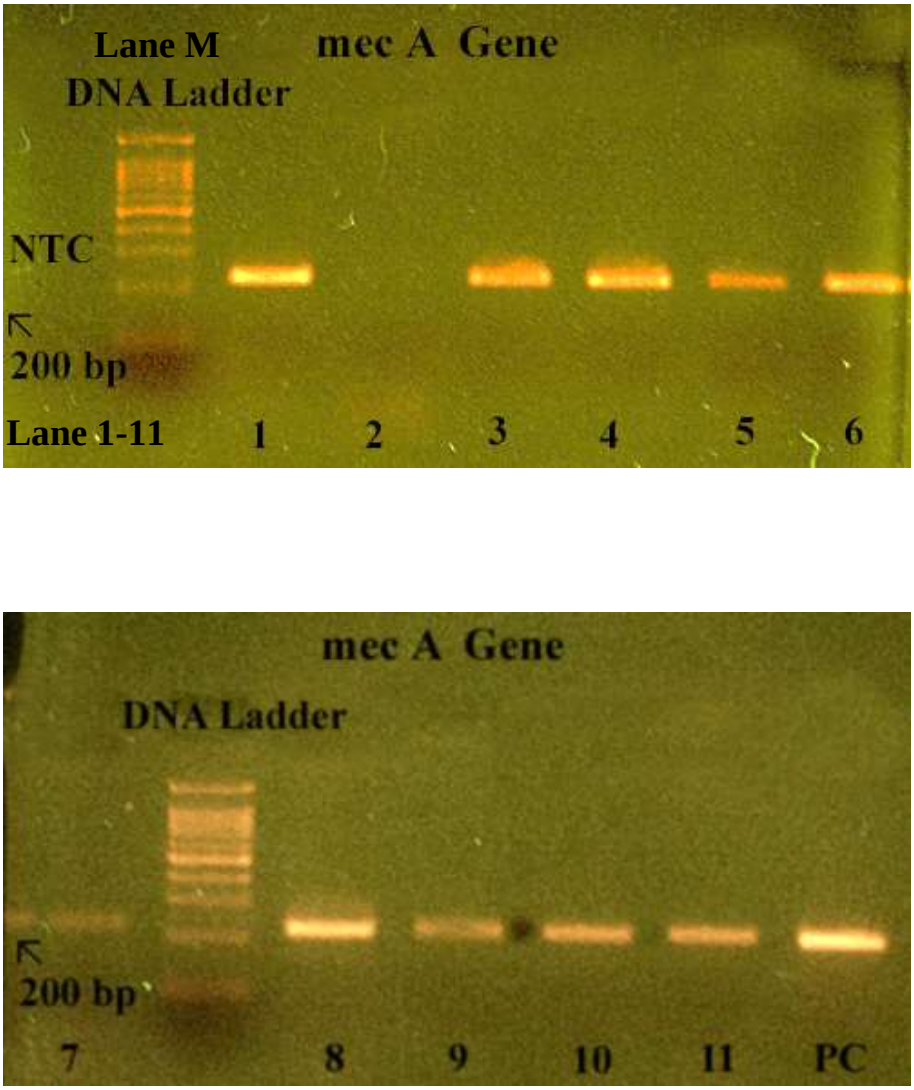


Figure 15: Agarose gel electrophoresis of PCR

Product (mec A gene)

NTC – Negative Template Control

Lane M shows DNA ladder molecular marker 100bp

Lane 1 – 11 shows MRSA strains and a positive control

Test sample showing 200bp gene products for mec A gene

Sample 2 – Negative for mec A gene

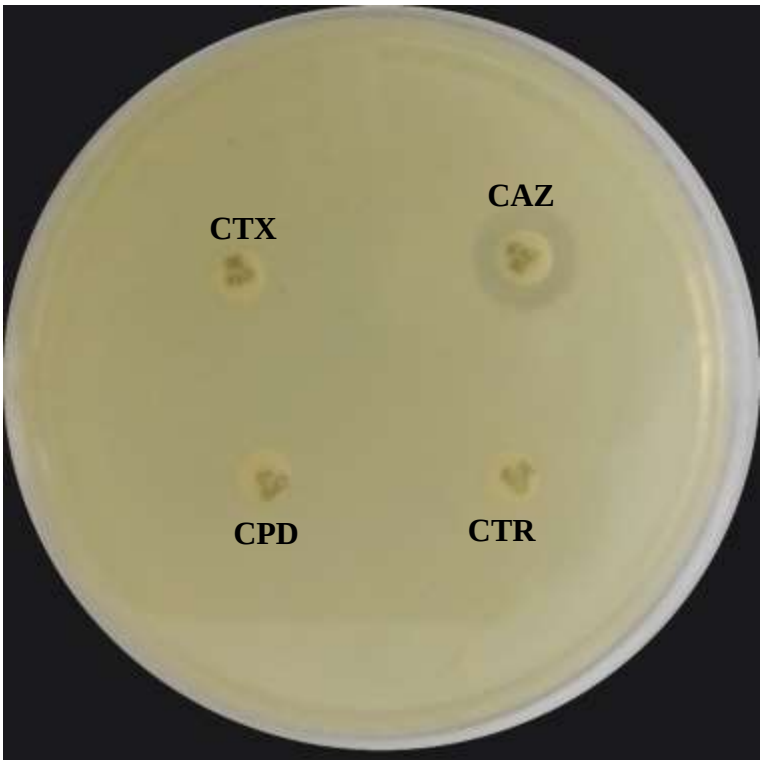


Figure 16: Phenotypic screening of ESBL by using 4 drugs

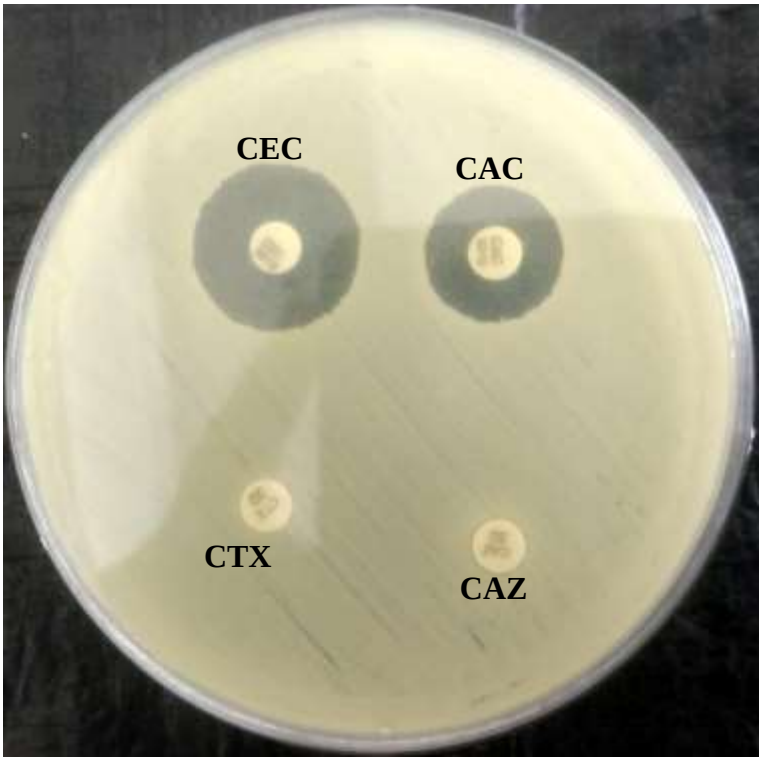


Figure 17: Phenotypic confirmation of ESBL by combined disc method

Figure 18: Fracture Tibia with Open Wound

External Fixator in Situ

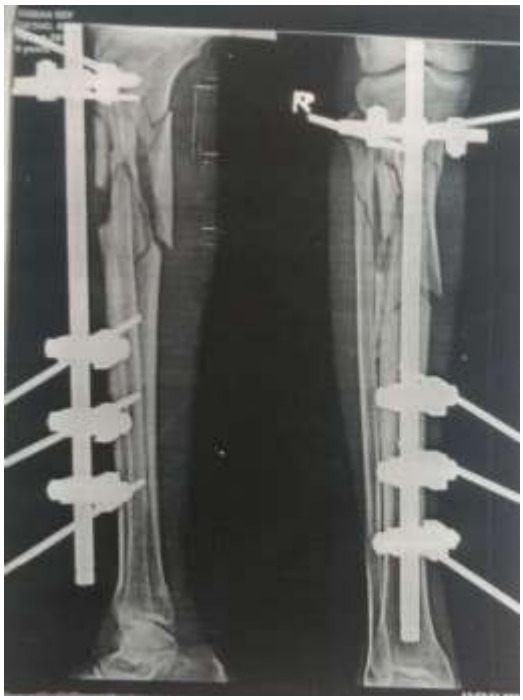


Figure 19: Implant – External Fixator

Figure 19: Implant – Dynamic Hip Screw

RESULTS

RESULTS AND ANALYSIS

A total of 137 orthopaedic device related infection (ODRI) cases were subjected for culture and sensitivity and their antibiotic resistance pattern were analysed in this study .

In a study period of one year (from july16 to june17), 1987 patients had undergone implant surgeries. Out of which 137 were clinically diagnosed as ODRI ' s and subjected for culture and sensitivity. Out of these , n = 116 (85%) were culture positive and n= 21 (15%) were culture negative . (Refer table no: 1) .

Among 116 culture positive cases , the nature of injury commonly observed was Road traffic accident n=84 (72%). The other causes of injury associated with ODRI ' s were self fall n= 24 (21%) , work spot injury n= 4 (4%), others (animal attack) n= 3 (2 %) and assault n=1 (0.8%) . Refer table no :2 .

Distribution of onset of infections was common in early/ acute infection n= 91(79 %) . Delayed infection was observed in n= 16 (13%) and chronic infection shows culture positive in n= 9 (8%) .(Refer table no: 3)

The percentage of culture positive in ODRI 's was observed more commonly in open fractures n= 82 (71%), and less commonly n= 34 (29%) in closed type of fractures. (Refer table no : 4).

In emergency surgery , the percentage of implant infection was observed in n= 74 (64%), and n= 42 (36%), in elective surgery cases. Refer table no: 5

The most common risk factor associated with implant infection was Diabetes mellitus n= 42 (36%) .Smokers and alcoholics developed implant infection in n= 26 (23%) and n= 25 (21%) respectively. Patients with anaemia developed infection in n= 9(8%) and those with chronic liver disease, chronic kidney diseases, systemic hypertension showed infection in n=8 (7%), and patients n= 6 (5%)developed implant infection even without any risk factor. (Refer table no: 6)

Out of 116 culture positives , implant infections were observed commonly in age group of 36-45 yrs , n= 35 (31%) . The next common age group involved in implant infections were 46-55 yrs ,n=23 (20%) and 26- 35 yrs , n=21(18%). Implant infections were low in 5-15 yrs n=5 (4%) .(Refer table no:7)

Implant infections were more common among males n=87 (75%) than females n=29 (25%) . Refer table no: 8 .

Out of 116 culture positive cases, Staphylococcus aureus was the most common pathogen 34(29%), followed by Klebsiella spp 21(18%), Pseudomonas aeruginosa 15(13%), Proteus spp 14(12%), CoNS 9(8%), E Coli 8(7%), Acinetobacter 3(3 %), Enterococcus fecalis 1(0.8%), and Polymicrobial 11(9%) (refer table: 9)

…….. A total of (n=34), staphylococcus aureus isolates showed 100% sensitivity to Linezolid and Vancomycin, 76% sensitivity to Cephazolin and 59% sensitivity to Amoxycilllin, Gentamicin, and Cephalexin, 56% sensitivity to Clindamycin, 50% sensitivity to Erythromycin and 46% sensitivity to Doxycycline. Cefoxitin sensitivity was 32% .Drugs such as Cotrimoxazole, Ciprofloxacin showed 15% sensitivity Refer table no : 10)

Among 34 Staphylococcus aureus, n=11 (32.3%) of the isolates were found to be MRSA (Methicillin resistant Staphylococcus aureus) and 23 isolates were MSSA(Methicillin sensitive Staphylococcus aureus).(Refer table number; 11)

All MRSA strains showed 100% sensitivity to Linezolid and Vancomycin . (Refer table no : 13)

Genotyping of all MRSA (n=11) strains were done, among the 11 resistant isolates, 10 (90.9%) resistant isolates showed mec-A gene positive. (Refer table no : 12)

Coagulative negative Staphylococcus species (n=9) showed 100 % sensitivity to Linezolid and Vancomycin . Amoxyclave showed sensitivity of 89% and Amoxycillin(77%). 50-60% sensitivity to Cotrimoxazole and Gentamicin Clindamycin showed sensitivity of 45%.(Refer table no:10)

One isolate of Enterococcus fecalis in this study showed 100% sensitivity to Amoxyclav, Ciprofloxacin, Clindamycin , Linezolid and Vancomycin. (Refer table no : 10)

All the gram negative bacilli and Pseudomonas in this study showed 100% sensitivity to Meropenum.(Refer table;13)

Klebsiella spp(n=21) showed 100% sensitivity to Amikacin, and sensitivity of 76% for Ciprofloxacin. Antibacterials such as Gentamicin, Cotrimoxazole, Amoxycillin, Cefotaxime, Ceftazidime, Piperacillin-Tazobactum, Cefaperazone-Sulbactum showed relatively less sensitivity.(Refer table:13)

Among the Psuedomonas aeruginosa(n=15) all the isolates showed 100% sensitivity to Amikacin, and 73% sensitivity to Ceftazidime. Sensitivity to Pipracillin-Tazobactum, was 67%,. Tobramycin showed sensitivity of 53% and Gentamicin, Ciprofloxacin showed sensitivity of 40-50%.(Refer table no:13)

Among the 14 isolates of Proteus spp, many of the isolates showed 80-90% sensitivity to Amikacin. Drugs such as Piperacillin-Tazobactum and Ciprofloxacin showed sensitivity of 64% and 50% respectively. Very low sensitivity to Cotrimoxazole, Amoxycillin, Gentamicin and Cefotaxime..(Refer table:13)

E Coli isolates(n=8) showed sensitivity of 80-90% to Ciprofloxacin, Amikacin. Drugs such as Amoxycillin, Cotrimoxazole, Gentamicin, Cephalexin, and Cefotaxime showed less sensitivity.(Refer table;13)

Acinetobacter spp(n=3), showed 100% sensitivity to Pipracillin-Tazobactum, and Meropenem. Amino glycosides such as Tobramycin showed 66% sensitivity and Amikacin 33% sensitivity(Refer table no: 13)

ESBL screening was done for Enterobacteriaceae isolates (Klebsiella spp, E Coli, Proteus spp) according to CLSI guidelines. In this study among the 21 Klebsiella spp isolates 10(47.6%) were suspected to be ESBL producer, among 14 Proteus spp, 7 (50%)isolates suspected to be ESBL producer, and among 8 E Coli isolates 5(62.5%) isolates found to be ESBL producer.(Refer table;14)

In this study, Phenotypic confirmation done for all the ESBL producing strains (n=22) according to CLSI guidelines by combined disk method. All the 22 strains were confirmed that, all are ESBL producers.(Refer table:14)

TABLES

TABLE NO:1 PERCENTAGE OF TOTAL CASES

PARAMETERS	NO OF CASES(n=137)	PERCENTAGE
TOTAL NO OF CULTURE POSITIVE	116	85%
TOTAL NO OF CULTURE NEGATIVE	21	15%
TOTAL NO OF CASES	137	100%

TABLE NO :2 NATURE OF INJURY IN CULTURE POSITIVE CASES.

NATURE OF INJURY	NO OF CASES(n=116)	PERCENTAGE
ROAD TRAFFIC ACCIDENTS	**84**	**72%**
SELF FALL	**24**	**21%**
WORK SPOT INJURY	**4**	**4%**
OTHERS(ANIMAL ATTACK)	**3**	**2%**
ASSAULT	**1**	**<1%**
TOTAL	**116**	**100%**

TABLE NO:3 DISTRIBUTION OF ONSET OF INFECTIONS.

Onset of Infection	No of cases (n=116)	Percentage
Early/Acute Infection	91	79%
Delayed infection	16	13%
Late/Chronic infection	9	8%
Total	116	100.0

p Value – 0.047 (Chi-Square test was used to calculate the p-value).

TABLE NO:4 DISTRIBUTION OF TYPE OF FRACTURE

TYPE OF FRACTURE	NO OF CASES(n=116)	PERCENTAGE
OPEN/COMMINUTED FRACTURE	82	71%
CLOSED FRACTURE	34	29%
TOTAL	116	100%

p Value – 0.049 (Chi-Square test was used to calculate the p-value).

TABLE NO :5 TYPE OF SURGERY

Type of surgery	No of Cases(n=116)	Percentage
Emergency surgery	**74**	**64%**
Elective surgery	**42**	**36%**
Total	**116**	**100%**

p Value – 0.042 (Chi-Square test was used to calculate the p-value).

TABLE NO:6 DISTRIBUTION OF RISK FACTORS

RISK FACTORS/CO-MORBID ILLNESS	NO OF CASES (n=116)	PERCENTAGE
DIABETES MELLITUS	**42**	**36%**
SMOKING	**26**	**23%**
ALCOHOLISM	**25**	**21%**
ANAEMIA	**9**	**8%**
SHT/CKD/CLD	**8**	**7%**
NO RISK FACTORS	**6**	**5%**
TOTAL	**116**	**100%**

TABLE NO: 7 AGE-WISE DISTRIBUSION OF CASES

AGE	NO OF ODRI(n=116)	PERCENTAGE
5-15 YRS	5	4%
16-25 YRS	20	17%
26-35 YRS	21	18%
36-45 YRS	35	31%
46-55 YRS	23	20%
55-70 YRS	12	10%
TOTAL	116	100%

TABLE NO;8 GENDER DISTRIBUTION OF CASES

Gender	No of Cases(n=116)	Percentage
Males	87	75%
Females	29	25%
Total	116	100%

TABLE NO :9 SPECTRUM OF ORGANISMS IN ODRI

Organism Isolated	No of Cases(n=116)	Percentage
Staphylococcus aureus	34	29%
Klebsiella spp	21	18%
Pseudomonas aeruginosa	15	13%
Proteus spp	14	12%
CoNS	9	8%
E Coli	8	7%
Acinetobacter spp	3	3%
Enterococcus fecalis	1	<1%
Polymicrobial	11	9%
Total	116	100%

TABLE NO :10 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE ORGANISMS

S.No	Antibiotics	Staphylococcus-auerus (34)	Coagulative – Negative-Staphylococcus(9)	Enterococcus-Faecalis (1)
1	Erythromycin(E)	17(50%)	2(22.2%)	-
2	Amoxycillin(AMX)	20(59%)	7(77%)	-
3	Doxycycline(DO)	15(44.%)	1(11.1%)	-
4	Gentamicin(GEN)	20(59%)	5(55.5%)	-
5	Co-trimoxazole (COT)	5(15%)	5(55.5%)	-
6	Ciprofloxacin(CIP)	5(15%)	-	1(100%)
7	Cephalexin(CN)	20(59%)	2(22.2%)	-
8	Cephazolin(CZ)	26(76.%)	-	-
9	Cefoxitin(CX)	11(32%)	-	-
10	Clindamycin(CD)	19(56%)	4(45%)	1(100%)
11	Linezolid(LZ)	34(100%)	9(100%)	1(100%)
12	Vancomycin(VAN)	34(100%)	9(100%)	1(100%)
13	Amoxycillin – clavulanic acid (AMC)	-	8(89%)	1(100%)

TABLE NO: 11 PERCENTAGE OF MSSA AND MRSA

S.aureus ISOLATES (n=34)	MSSA PERCENTAGE	MRSA PERCENTAGE
34	23 (67.7%)	11 (32.3%)

TABLE NO: 12 GENOTYPING – PERCENTAGE OF mecA GENE

MRSA STRAINS (n=11)	GENOTYPING BY PCR	
	Percentage of mec A gene Positive	Percentage of mec A gene Negative
11	90.9%	9.1%

TABLE NO:13 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE ORGANISMS

S.No	Antibiotics	Klebsiella-Species (21)	Pseudomonas-Aeruginosa (15)	Proteus-Species (14)	E.coli (8)	Acinetobacter (3)
1	Amoxycillin (AMX)	5 (23.8%)	-	1 (7.1%)	2 (25%)	-
2	Cotrimoxazole (COT)	5 (23.8%)	-	1 (7.1%)	1 (12.5%)	-
3	Gentamicin (GEN)	10 (48%)	7 (47%)	3 (21.4%)	2 (25%)	-
4	Amikacin (AK)	21 (100%)	15 (100%)	12 (85.7%)	7 (87.5%)	1 (33%)
5	Ciprofloxacin (CIP)	16 (76%)	7 (47%)	7 (50%)	7 (87.5%)	-
6	Cephalexin (CN)	3 (14.2%)	-	-	1 (12.5%)	-
7	Cefotaxime (CTX)	6 (28.5%)	3 (20%)	5 (35.7%)	2 (25%)	-
8	Ceftazidime (CAZ)	2 (9.5%)	11 (73.%)	7 (50%)	3 (37.5%)	-
9	Piperacillin-Tazobactum (PIT)	3 (14.2%)	10 (67%)	9 (64%)	1 (12.5%)	3 (100%)
10	Meropenem (MRP)	21 (100%)	15 (100%)	14 (100%)	-	3 (100%)
11	Tobramycin (TOB)	-	8 (53%)	-	-	2 (66%)

TABLE NO:14 PERCENTAGE OF ESBL

ISOLATES	NO OF ISOLATES	COMBINED DISC TEST POSITIVE	PERCENTAGE OF ESBL
E.COLI	8	5	62.5%
PROTEUS	14	7	50%
KLEBSIELLA	21	10	47.6%

CHART 1: DISTRIBUTION OF CULTURE SENSITIVITY

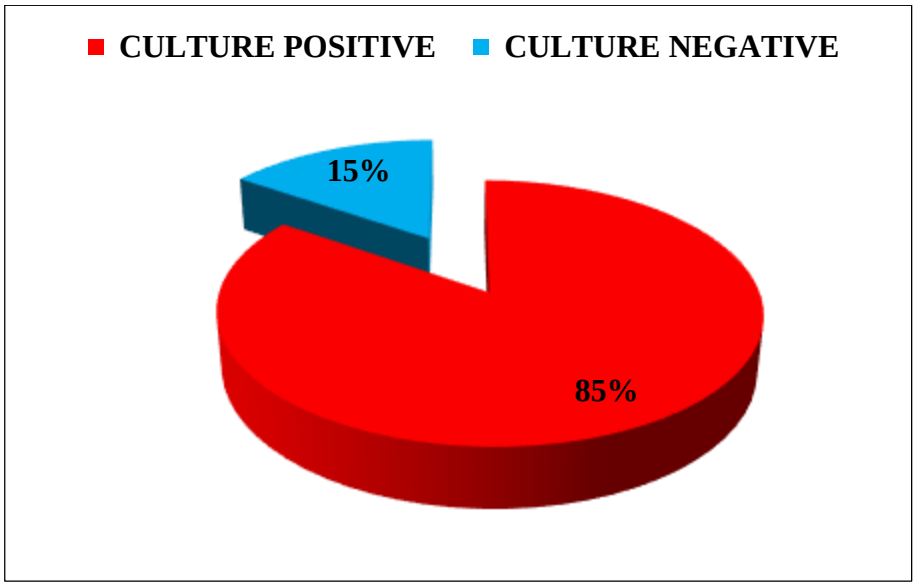


CHART 2: NATURE OF INJURY

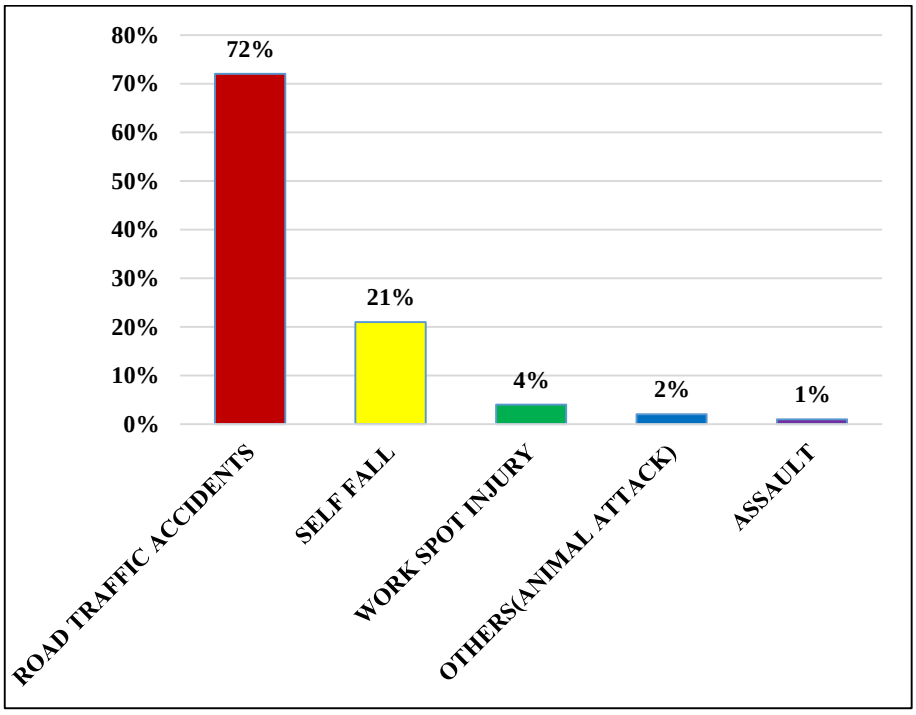


CHART 3: ONSET OF INFECTION

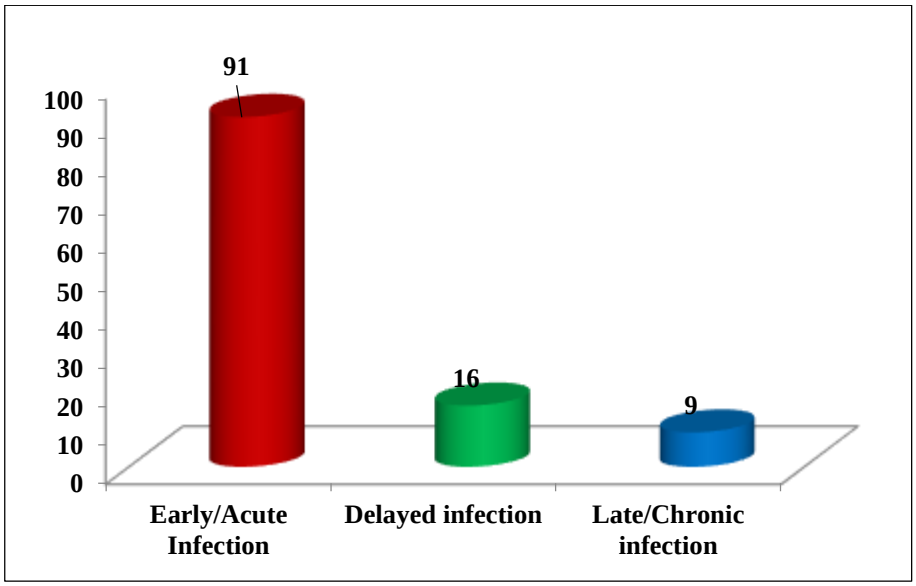


CHART 4: TYPE OF FRACTURE

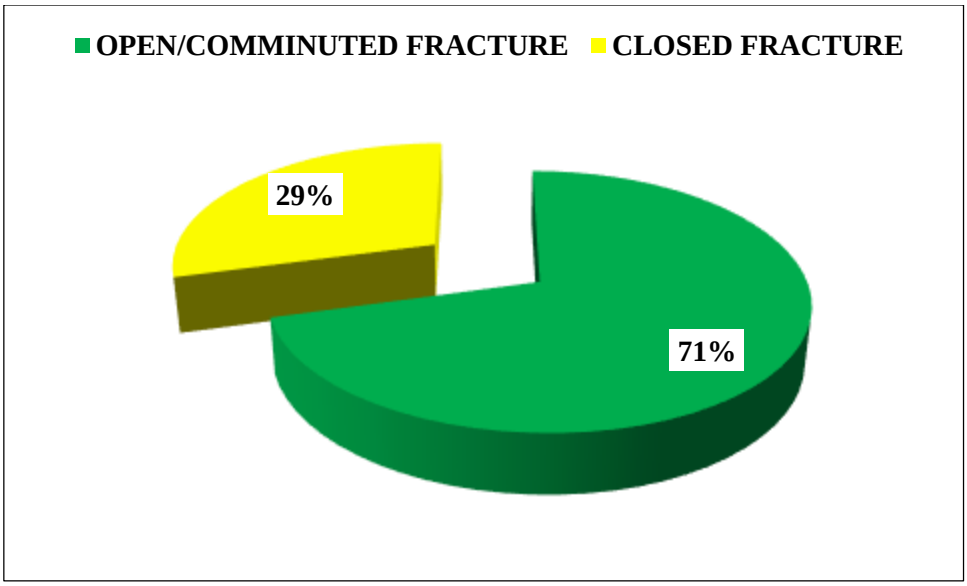


CHART 5: TYPE OF SURGERY

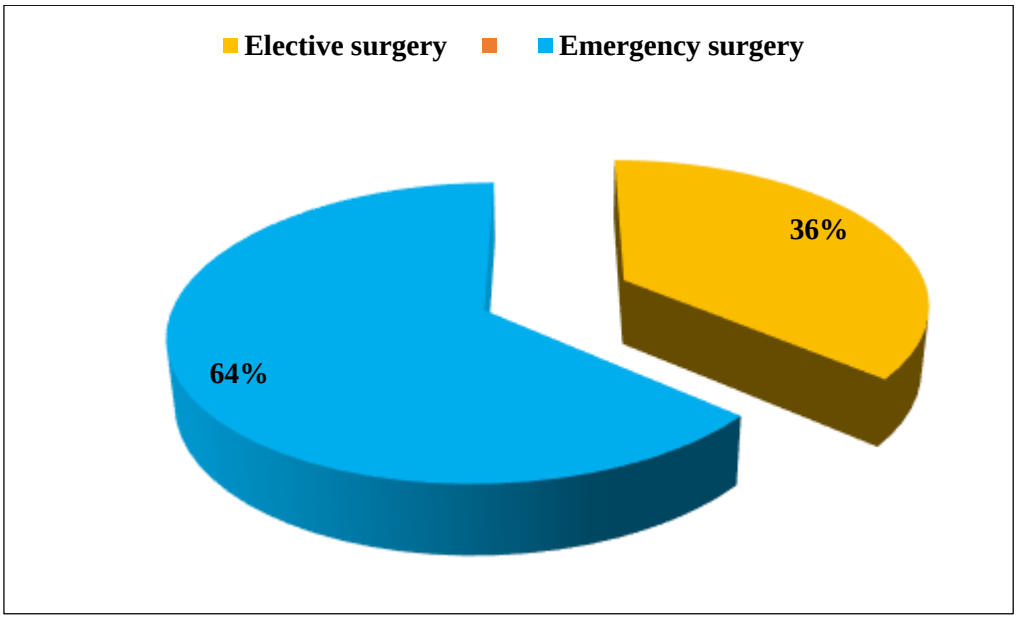


CHART 6: DISTRIBUTION OF THE RISK FACTORS

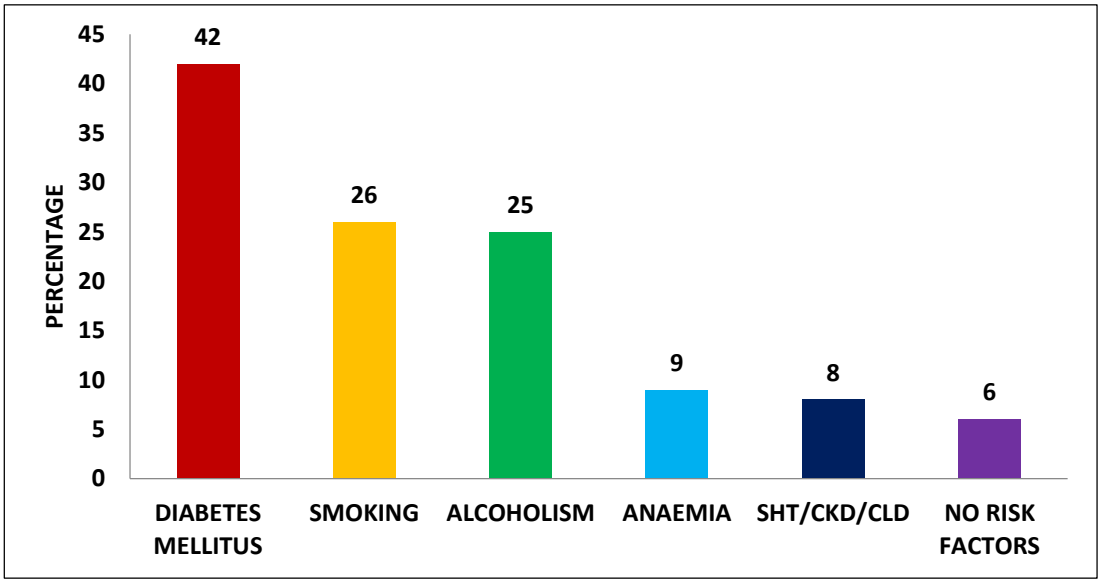


CHART 7: AGE-WISE DISTRIBUTION OF THE CASES

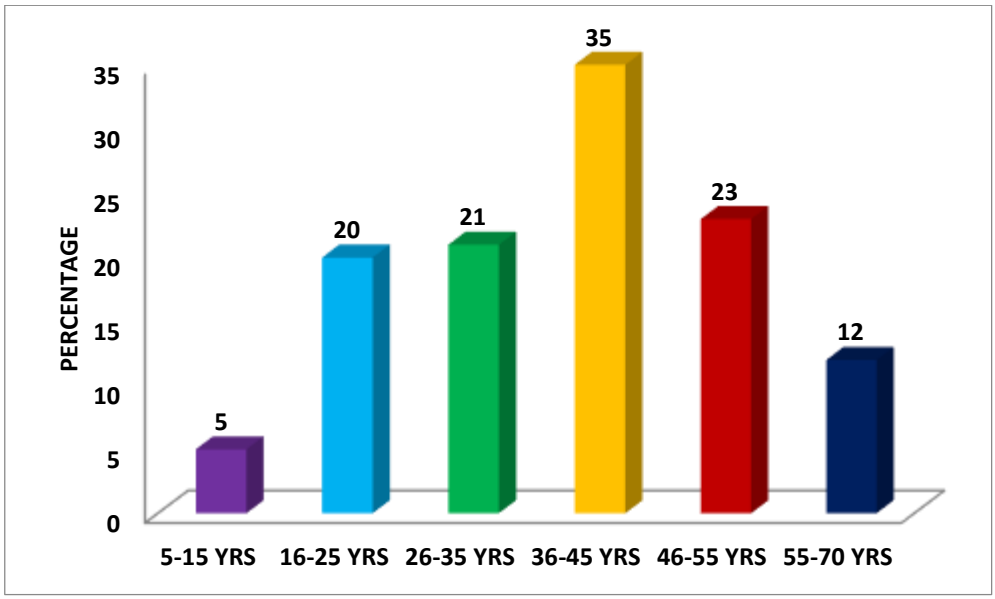


CHART 8: GENDER-WISE DISTRIBUTION OF THE CASES

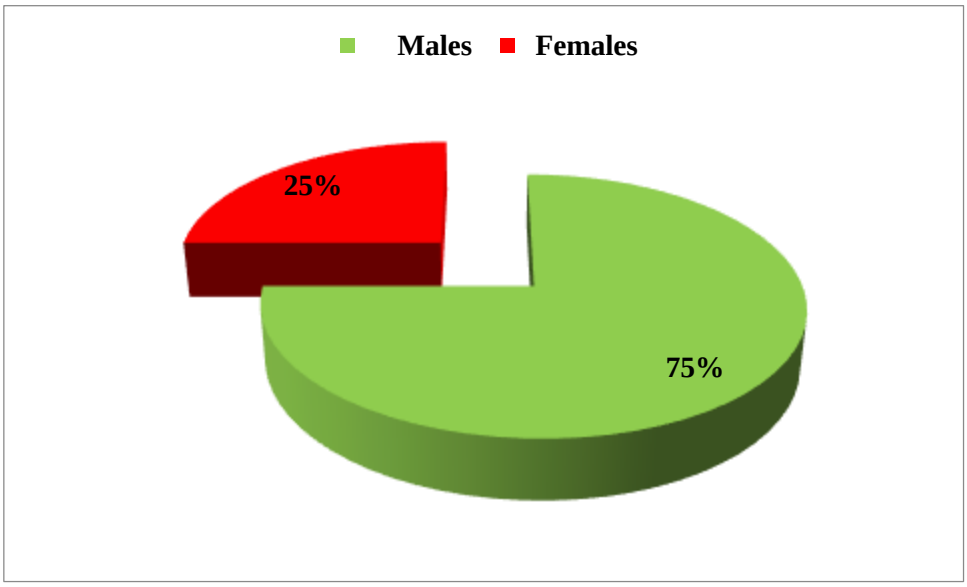


CHART 9: SPECTUM OF ORGANISMS IN ODRI

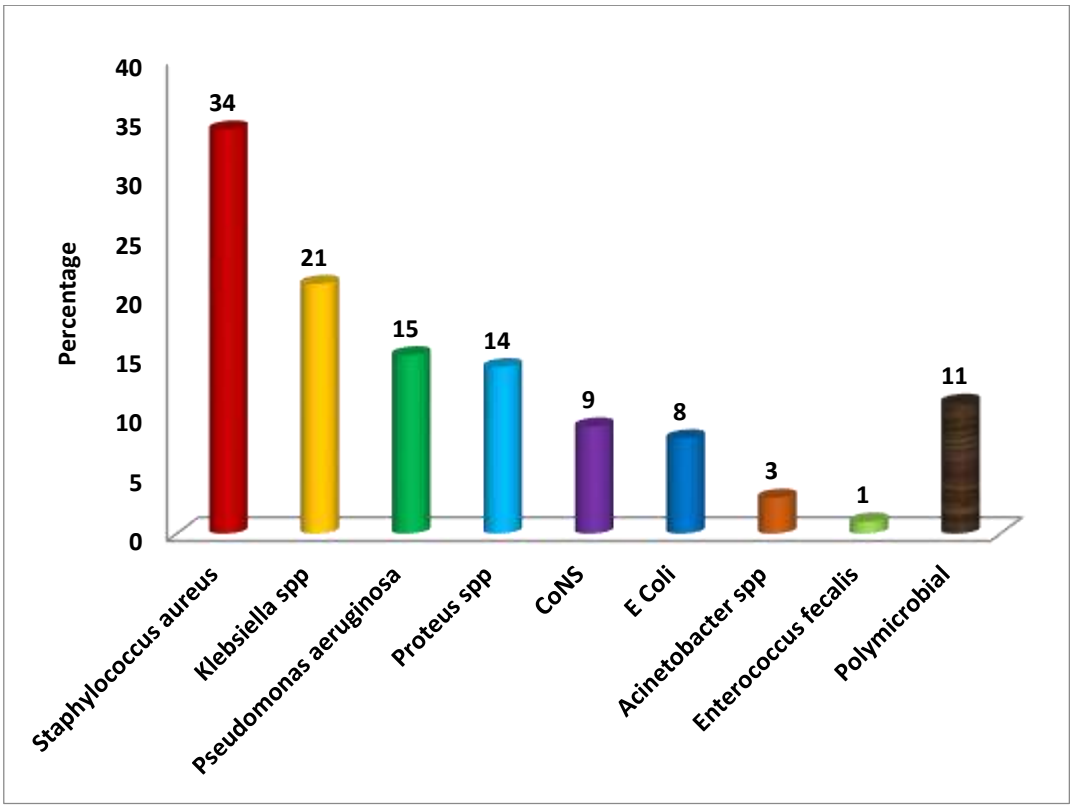


CHART 10: PERCENTAGE OF MSSA AND MRSA

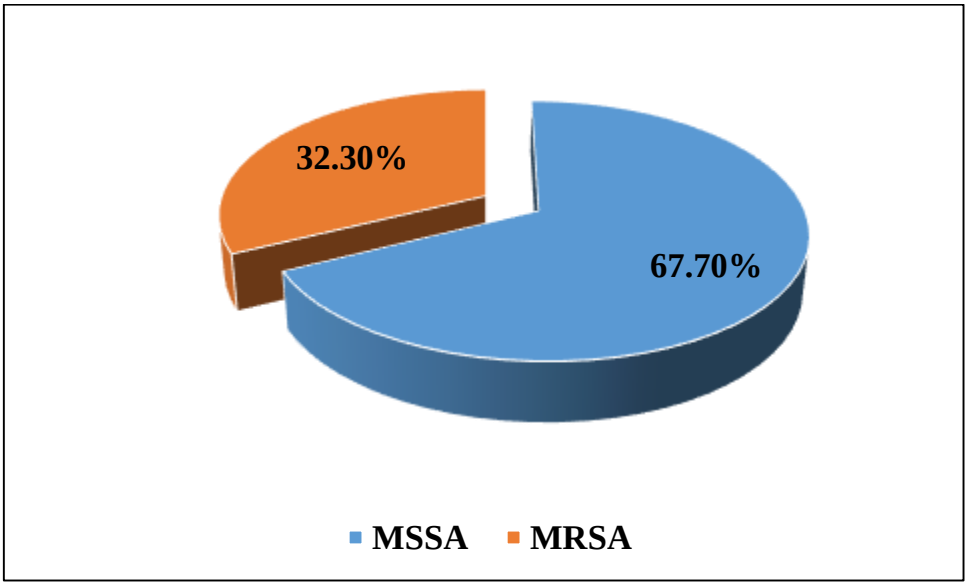


CHART 11: GENOTYPING – PERCENTAGE OF mec A gene

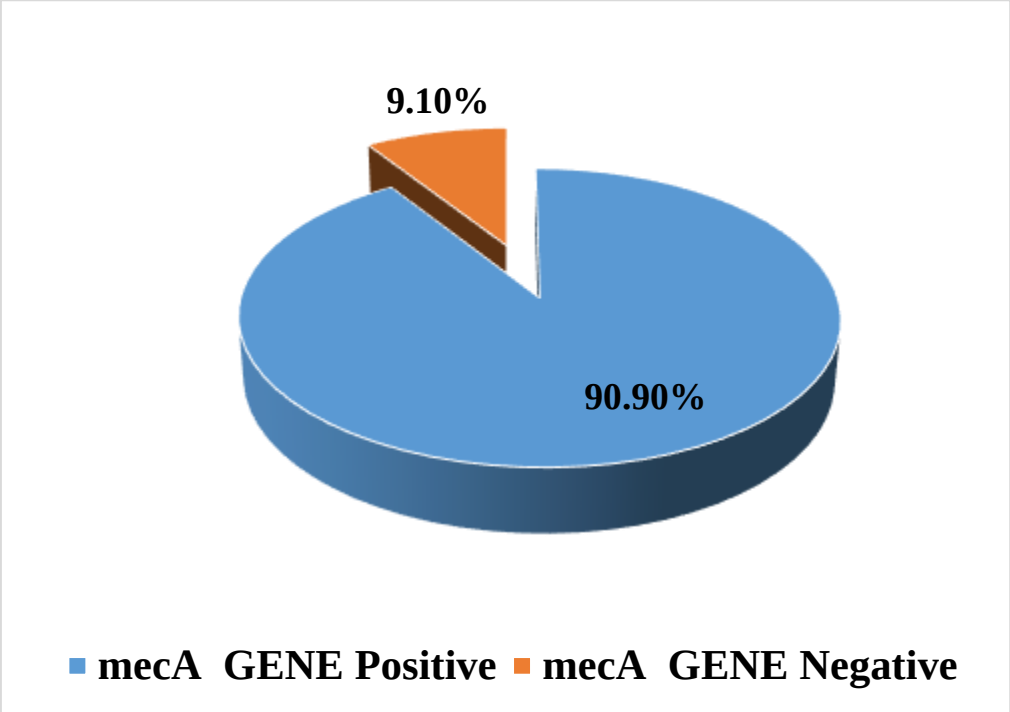
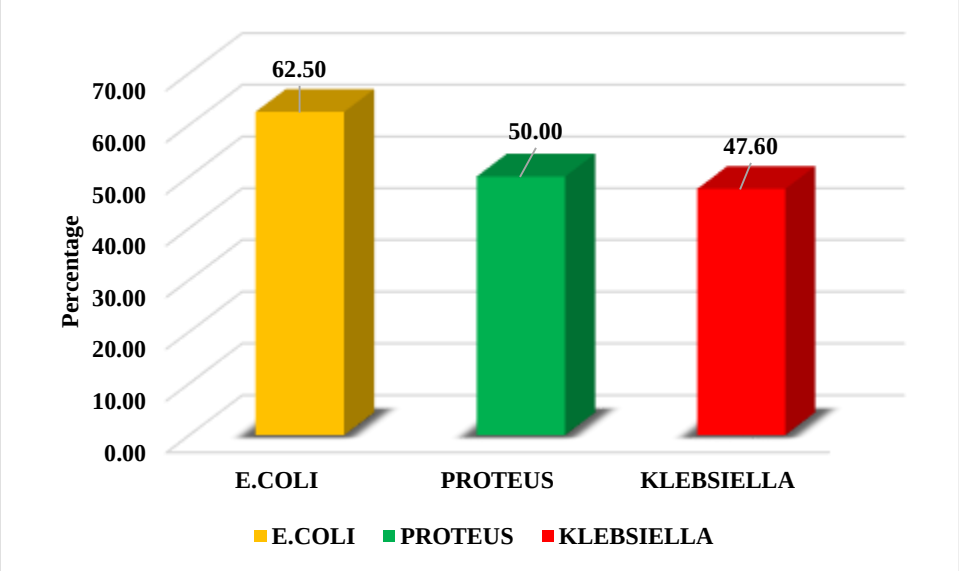


CHART 12: PERCENTAGE OF ESBL



DISCUSSION

DISCUSSION

This study was conducted on patients with orthopaedic implant infections admitted in orthopaedic and plastic surgery wards, Coimbatore Medical College Hospital, Coimbatore. Of the 1987 patients who underwent orthopaedic implant surgeries for bone fractures and joint replacement, 137 patients developed implant infection and they were included in this study. The purpose of this study is to isolate the bacterial pathogens causing implant infection and to know their resistance pattern with reference to Methicillin sensitivity/resistance and ESBL production.

The isolates and the pattern of their susceptibility and resistance to the antibiotics are continuously changing. So periodic survey of isolation and the susceptibility pattern of isolates, in patients with implant infections become essential.

INCIDENCE OF INFECTION:

Of the 1987 patients who underwent orthopaedic implant surgeries for bone fractures and joint replacement, 137 patients developed implant infection (6.8%) , which correlates with the study by Ravikanth das et al(2015) , Angappan perumal et al (2016)[20,21]. Among 137 cases, 116(85%) have been culture positive and 21(15%) cases were culture negative, which correlates with the study by Trupti B .Naik et al (2016). [23]

Most of the studies specify that the currently incidence of ODRI's should be < 1-2 % . But the incidence of culture positivity in

the present study is relatively higher than the accepted standards .The higher percentage of infection in our study could be attributed to the following reasons:

1) Majority of the patients were operated due to trauma, and it has been reported that pre-operative soft-tissue damage is a major risk factor for developing ODRI
2) The other important risk factors for developing ODRI is an emergency operation.
3) In our study, more number of patients had undergone emergency surgery

In contrast , low incidence of implant infection(2.55%) was observed by Fahed A. Al-Mulhim et al in his study (2014) [22].

NATURE OF INJURIES:

Fractures following RTA(road traffic accident) constitutes about 72% , of the total 116 culture positives were encountered in this study that correlates with the study of Angappan perumal et al (2016), Anisha fernandes et al (2013).[21, 24]

The percentage of implant infection was high in RTA , as this causes extensive soft tissue injury , wound contamination and devascularisation of periosteum following fractures that leads to infections. [24, 25]

ONSET OF INFECTION:

ODRI's showed culture positivity in 79% of acute/early onset (< 3months after surgery) cases and 13% in delayed infection and 8% in chronic infection in the present study . Similar incidence was quoted by A.D Koshravi et al 2009(72.9%, 22.6%, and 4.5% respectively) . [26]

This high prevalence of early infection may be related to

1. Inadequate disinfection procedures to eliminate microorganisms from the environment,
2. Contamination of surgical instruments and /or contaminated implants.
3. In addition to that, trauma and fracture fixation using metallic implants may produce structural and functional damage to the local host tissue resulting in impaired humoral and cellular immune response.

On a local level this may decrease resistance to the pathogenic microbial load with subsequent manifestation of infection in the traumatized tissue and put the patients at a higher risk of early infection .

Type of fracture:

Out of 116 cases, 82 (71%) had open fracture and 34 (29%) had closed fracture. The incidence of infection was more in open fracture when compared to closed fracture that correlates with the study of Prakash Doshi et al (2016), and Angappan perumal et al (2016) .[27, 21]

The cause for increased infection in open fractures can be attributed to the fact, that skin is normally a barrier to outside contaminants,

indwelling organisms . However when the skin is broken at the time of injury, bacteria both from skin of the individual and outside environment can easily travel down to the broken bone and cause infection. This is the basics in pathophysiology of open fractures.

IMPLANT INFECTIONS BASED ON THE TYPE OF SURGERY:

Out of 116 cases , 74 (64%) had undergone emergency surgery and 42 (36%) cases had undergone elective surgery . In this study it was found that ODRI was more common in patients who have undergone emergency surgery as compared to less incidence of ODRI in elective surgery that correlates with study of Ta Wei Kevin Kok et al (2016) . [28] Higher rate of infection in emergency surgery was due to various factors such as the following:

1. poor general condition of patient,
2. contamination at the surgical site, less preparation time for surgery, inadequate preoperative optimisation of co-morbidities(anaemia, diabetes etc) and not giving any prophylactic antibiotics. Ta Wei Kevin Kok et al (2016)in his study, he observed that increased incidence of ODRI was in emergency implant surgeries.

Risk factors:

Diabetes mellitus n= 42(36%) was considered as an important risk factors for ODRI, which was comparable to the study by Angappan Perumal et al (2016) , Ta Wei Kevin Kok et al (2016) .[21, 28]He also He also

noted in his study that prolonged surgery time was another risk factor for ODRI. In our study, in addition to diabetes , alcoholism and smoking were also noted as risk factors for ODRI's.

The pathogenesis of Diabetes in causing wound infection in implant surgeries is mainly due to the hyperglycaemic state that impairs neutrophil chemotaxis and phagocytosis, resulting in weakened antibacterial defence and impaired wound healing. Suboptimal glucose control peri-operatively is associated with increase in the length of the hospital stay, so optimising blood sugar level peri-operatively becomes essential to decrease the postoperative adverse outcome.

AGE-WISE DISTRIBUTION OF CASES:

In this study, patients were divided into six different age groups. Out of 116, a maximum of 35(31%) patients are in 36-45 years age group, This high percentage of implant infections in this age group mainly due to :

1. More number of actively working population (36-45 years)
2. Young adults are more involved in RTAs, they exhibit increased risk behaviour for RTA(drunken driving, rash driving etc)

It is comparable to that of study by Angappan Perumal et al(2016), who showed high incidence of ODRI in 21-60 years age. [21]

GENDER DISTRIBUTION OF CASES:

In the 116 infected cases, 87 (75%) patients were male and 29 (25%) were females. This high male predominance can be attributed to,

1) Men are more prone for trauma because they travel outside for work frequently such as to construction sites, industries, factories etc

Our study correlates with the study conducted by Angappan Perumal et al(2016), who have recorded 85.96% male and 14.03% females and Muhammad Salman et al (2014). [21 , 29]

CORRELATION BETWEEN ANTIBIOTIC PROPHYLAXIS AND ODRI's :

The patients with preoperative antibiotic prophylaxis developed less implant infection (32%) when compared to those without antibiotic prophylaxis which was (62%) that correlates with the study of Muhammad Shoaib Khan et al (2008) . In contrast the study by Trisha N.Peel et al (2012) showed the percentage of implant infection was high in patients even with antibiotic prophylaxis (63%) , which could be due to the local prevalence of wide spectrum of pathogens involved in ODRI' s . [32, 31]

POLYMICROBIAL INFECTION:

Among 116 culture positive cases, 105(90.5%) isolates were mono microbial and 11(9.5%) were poly microbial. Study by Juan C. Martinez-Pastor et al (2009), showed 59.5% of polymicrobial. Another study by Trisha N.Peel

et al (2012) showed Poly microbial infection(36%) frequently involved combination of Gram negative and Gram positive organisms.

In our study commonest association was found between Staphylococcus aureus and Klebsiella species. [30, 31] The reasons for polymicrobial in ODRI could be due to:

a) Human endogenous flora contaminating the wound frequently causes poly microbial infection.
b) The biofilm forming organisms were commonly associated with polymicrobial infections .

SPECTRUM OF ORGANISMS IN ODRI 's :

Out of 116 culture positive cases, Staphylococus aureus was the most common pathogen isolated 34(29%), followed by Klebsiella spp 21(18%), Pseudomonas aeruginosa15(13%), Proteus spp 14(12%), CONS 9(8%), E Coli 8(7%), Acinetobacter 3(3%), Enterococcus fecalis 1(<1%) and polymicrobial 11(9%).

This correlates with the study of Roopa shree et al, in 2015 at Bangalore [25] and Dr Lakshmi narayana et al, in 2013.

In this study, Gram positive cocci S. aureus was the most common isolate , followed by Gram negative bacilli Klebsiella spp. This mainly supports an intra operative contamination and assumed that these were the main nosocomial pathogens in the operating room.

GRAM NEGATIVE BACILLI:

In our study, among the 61 isolates of gram negative bacilli, 21 klebsiella species, 15 Pseudomonas species, 14 Proteus species, 8 E.coli species and 3 Acinetobacter species were isolated. Majority of the gram negative bacilli in our study showed 100% sensitivity to Meropenem and 80-90% sensitivity to Amikacin, 60% sensitivity to ciprofloxacin, Cefaperazone-sulbactam and Piperacillin-Tazobactam.

This correlates with the study of Ravi kant das et al, and Anirudh dash et al in 2015 which showed a similar sensitivity pattern. Gram negative bacilli are common in Implant infections which could be due to

1. Prolonged stay in the hospital wards
2. Cross-infection.[32]

Anaerobes: Though anaerobes play a significant role in the pathogenesis of implant infections, no anaerobes were isolated in this study, the reason could be a) anaerobes usually involved in late/ chronic infections(>24 months), in this study, only less number of cases (9 cases) were of chronic infection.b) antimicrobial therapy should be stopped at least 2 weeks prior to the tissue sampling for anaerobic cultures. But most of the patients were already on an empirical therapy or they had a history of an antimicrobial treatment in the recent past. Other reasons may be need better sample collection method by avoidance of swabs, inoculation of multiple plates, and longer incubation period for better isolation of anaerobes.

Gram positive bacteria showed 100% sensitivity to Linezolid and Vancomycin and 76% sensitivity to Cephazolin .

All all the Gram negative bacilli and Pseudomonas showed 100% sensitivity to sensitivity to Meropenem .

The most common Gram positive bacteria isolated in this study was Staphylococcus aureus 34(29%) which showed 100% sensitivity to Linezoild, and Vancomycin. Cefazolin showed sensitivity of 76%. Cefoxitin sensitivity was 32%. Drugs such as Ciprofloxacin, Cotrimoxazole showed only 15% sensitivity.

S.aureus was higher in our study which could be due to

1. Intra-operative contamination
2. Inoculation of skin where, S.aureus is a commensal.[34]

Coagulative negative Staphylococcus species (n=9) showed 100% sensitivity to Linezolid, and Vancomycin. Enterococcus fecalis (n=1) isolated in this study showed 100% sensitivity to Amoxycillin-Clavulanic acid, Ciprofloxacin, Clindamycin, and Vancomycin.

MRSA isolated in this study was 11(32.3%) and MSSA 23(67.7%). This correlates with the study by Goel et al (2013). In his study, he observed that MRSA was 30%, but in contrary, study by Satya Chandrika V et al(2016) showed MRSA was about 64%.

The best evidence for antibiotic selection is available for Staphylococci. All MRSA strains showed 100% sensitivity to Linezolid and Vancomycin. In case of Rifampicin resistance, Moxifloxacin can be used as monotherapy. In case of MRSA, alternative to beta-lactams, Vancomycin or Daptomycin, may also be used and is well tolerated. Rifampicin is of crucial importance in the treatment of ODRI, as an anti-Staphylococcal biofilm antibiotic, and has been associated with a high rate of treatment success. But Rifampicin should not be given as mono therapy due to rapid development of drug resistance. Initially it is administered along with a bêta-lactam antibiotic, later combined with a quinolone especially with Ciprofloxacin or Levofloxin.

Study by T. Fintan Moriarty et al(2016) reveals that in case of quinolone resistance, Rifampicin can be given along with Fusidic acid, Cotrimoxazole, Linezolid, Clindamycin or Minocycline.

Local antibiotic delivery:

It has been described since 1970s, certain biomaterial may be used as carriers or vehicles, for the delivery of antibiotic agents to the site of infection and of late it also has become a regular adjunct in the treatment of ODRI. Local delivery of drugs has many advantages over systemic delivery, which can offer for the potential for significant supportive antimicrobial effect. Since the antibiotic is placed directly at the site of interest, an intact vascular

system is not required to reach the surgical site, which may be particularly useful in trauma patients.

Local delivery can also achieve local concentrations much higher than those achievable systemically, thereby not only improving local concentration, but reducing the risk of systemic toxicity. Bone cement is a ideal vehicle for antibiotic delivery and Gentamicin was identified as suitable antibiotic due to the fact that it was found to withstand the elevated temperatures of curing bone cement. Antibiotic- loaded bone cements are useful in improving ODRI outcomes.

New approaches for prevention and treatment:

1) **Active and passive vaccines**: based on its cost effectiveness, vaccination is an important approach to prevent, treat and potentially eradicate ODRIs. But unfortunately, all efforts to develop an effective vaccine against S. aureus have failed for a number of reasons. The main reason is that S. aureus has co-evolved with mammalian hosts to become a human commensal. Thus all patients have some level of acquired immunity against S.aureus prior to surgery. Measurement of the immune response against S. aureus may help guide future prophylaxis and therapy.
2) **Silver**: Silver is a potent candidate for coating devices, as it gives a broad spectrum of antibacterial activity against planktonic and sessile Gram positive, Gram negative and multi drug- resistant bacteria.

3) **Antimicrobial and anti-biofilm peptides**: Antimicrobial peptides(AMPs) are innate defence molecules of animals, plants and microorganisms, with a broad spectrum of antimicrobial activity and low risk of resistance development in general.

4) **Quorum- sensing inhibitors**: Quorum sensing is a mechanism that many microorganisms use to coordinate gene expression in response to local conditions, including cell density. Combining conventional antimicrobial agent with a quorum sensing inhibitor might circumvent the problem of biofilm tolerance.

5) **Biofilm degrading enzymes**: Anti-biofilm strategy depends on the use of biofilm-degrading enzymes, and both deoxyribonuclease1(DNase 1)and exopolysaccharide-degrading dispersion B(DspB), could have applications in the prevention or treatment of biofilm infections associated with orthopaedic implants.

   All these emerging technologies and interventions may be expected to improve in the successful treatment of ODRIs in future.

Phenotyping of Methicillin resistance Staphylococcus aureus was done using Cefoxitin(30µg) disc according to CLSI guidelines. Genotyping by PCR was done for all MRSA strains to identify Staphylococcal chromosomal cassette (SCC)mec elements that contains chromosomal gene mecA which encodes for penicillin binding protein 2A(PBP2A).

Out of 11 MRSA isolates, 10 isolates (90.9%) were positive for mecA gene. A single isolate was negative for mecA gene and could be due to the presence of other resistance mechanisms such as

1) large amounts of beta-lactamase or

2) lack of optimal PCR conditions or

3) change in mecA gene due to mutations.

Mec A gene detection by PCR method is the gold standard for identifying methicillin resistance in S.aureus isolates. Mec A gene is present in MRSA strains which encodes penicillin binding proteins (PBP 2a). MRSA has become a major public health problem all over the world. It is correlated with increased morbidity, compared to other bacteria.

Mec A gene was found in 10 out of 11 MRSA isolates in our study.

In a health care setting MRSA infections can cause severe problems in a hospital or nursing home, such as blood stream infections, pneumonia, and surgical site infections. MRSA infections, if not diagnosed and treated early can cause severe sepsis and mortality.

MRSA is usually spread by (1) direct contact with a patient with MRSA infection or colonisation

2) Hand hygiene - Not following proper hand washing and use of PPE

3) From a health care personal / Nasal carriage.

Also nasal carriage of MRSA in people who do not have signs and symptoms of infection, can spread the bacteria to others. Studies shows that about 33% of people are nasal carriers of MRSA. CDC study in 2011, also showed morbidity and mortality due to MRSA isolates.

CDC guidelines to combat antibiotic resistance plan for MRSA:-

1. To know the type of drug - resistant infections present in your facility and patients.
2. Request immediate alerts when the laboratory identifies drug-resistant isolates.
3. Alert the other facility when you transfer a patient with drug-resistant isolates.
4. Follow relevant guidelines and precautions at every patient encounter like appropriate handling of patient care, equipment / devices.
5. Follow appropriate antibiotic policy.
6. Removal of temporary medical devices as early, especially when it is not needed.

SUMMARY

SUMMARY

The present study was carried out in the Department of Microbiology in collaboration with the Department of Orthopaedics, Coimbatore Medical college, Coimbatore for a period of one year from July 2016- June2017.

In this study, 116 culture positive isolates were identified including Gram positive cocci and Gram negative bacilli from orthopaedic implant infections. MRSA strains and ESBL production by Gram negative bacilli were detected by phenotypic methods and molecular characterization by PCR for MRSA strains.

1) A total of 137 clinically suspected cases of orthopaedic device related infections(ODRI's) were studied in all age groups, and in both sexes irrespective of pre- operative administration of antibiotics in patients who had undergone implant surgery.
2) Out of 137 ODRI's , culture positivity was found in 85% .
3) Age predilection for ODRI was found to be common in 36-45 year age group.
4) ODRI 's were predominantly seen among males than females in a ratio of 2.9 : 1.
5) Among the nature of injuries, more number of cases(68%) were due to road traffic accidents(RTA) .
6) Regarding the onset of infections, majority of the patients(76.6%) had early/acute onset of infection .

7) In the present study implant infection with open fracture was found to be high (68.6%) as compared to closed fracture .

8) The present study reveals , those who had undergone emergency surgery (64%) had developed implant infections more , when compared to elective surgery(36%) .

9) The most common risk factor was found to be diabetes mellitus (36%) followed by smoking (23%) and alcoholism(21%).

10) Out of 116 patients , the culture positivity developed by patients with preoperative antibiotic prophylaxis was less (38%) when compared to those without antibiotic prophylaxis which was more (62%) .

11) Among the 116 culture positive cases, 105 isolates were monomicrobial and 11 isolates showed polymicrobial growth.

12) Among the 105 monomicrobial isolates, 44 isolates were found to be Gram positive cocci, and 61 isolates were found to be Gram negative bacilli.

13) Staphylococcus aureus is the most common Gram positive cocci 34(77.2%) isolated and Klebsiella spp is the most common isolate among Gram negative bacilli 21(34.4%).

14) Out of 34 isolates of Staphylococcus aureus 11 isolates were found to be MRSA strains which is about 32.3%

15) ESBL screening was done for all Klebsiella spp, Proteus spp, and E Coli isolates. Out of 61 Gram negative isolates, 22 isolates

(Klebsiella-10, Proteus-7, and E Coli-5) were found to be ESBL producers.

16) Phenotypic confirmation test was done for all the 22 ESBL by combined disk diffusion method and all 22 strains were found to be ESBL producers.

17) Genotyping done for all 11 MRSA strains, out of 11 MRSA strains 10 isolates found to be positive for mecA gene.

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CONCLUSION

CONCLUSION

Orthopaedic device related infection (ODRI) is a diagnostic and therapeutic challenge which can pose a serious threat to the patient leading to high morbidity. This study has given us a wide knowledge about ODRI and their incidence in our hospital and also helps us in finding out the bacteriological profile of organisms causing implant infection and their sensitivity pattern.

The infection rate in primary orthopaedic surgery as found in our study is comparatively high and so there is need for proper measure of infection control as it has great financial burden on patient and on hospital resources and could lead to increased morbidity and mortality in patients.

While analysing risk factors , Diabetes mellitus was the most common co-morbid entity for ODRI's, hence preoperative glycemic control is essential before planning for surgery. Next to diabetes , Alcoholism and smoking were considered to be a definitive risk factor for developing implant infection.

Among the culture positive cases, Staphylococcus aureus was found to be most common Gram positive isolate and Klebsiella species as the predominant Gram negative isolate.

Treatment of ODRI is a scenario where many variables play a role in deciding the treatment. The treatment however is difficult and prolonged. Therefore, the best treatment strategy is prevention of infection. Antimicrobial

prophylaxis remains the single most effective method of reducing the prevalence of infection.

Treatment of ODRI requires prolonged antibiotic treatment of a minimum of 2 weeks upto 6 months. The choice of the antibiotic regimen depends on duration and pathogenesis of infection, stability of the implant and antimicrobial susceptibility of the pathogen.

In most instances, empirical treatment is being given for ODRI cases. Inappropriate and misuse of antibiotics can cause multidrug resistance to commonly used antibiotics. Thus usage of antibiotics should be based on local and current trends on prevalent pathogens and its sensitivity pattern.

This study clearly shows the changing trends of microbial isolates with special emphasis on the emergence of MRSA and ESBL strains in our hospital set-up. The diagnostic microbiology thus plays a crucial role in this context and aids the surgeons in the proper selection of antibiotics as per the Antibiotic policy guidelines issued by Hospital Infection Control Committee.

With the evolution of newer techniques such as arthroscopy, recent advances in trauma and fracture management, introduction of the modern arthroplasty, and spine surgeries, the risk of infection is a great threat. We all know that implant infections and osteomyelitis might just be the most difficult morbidities to treat and patients with osteomyelitis may even land up in amputations. Hence It is always better to prevent the development of implant infections and take appropriate to prevent ODRI.

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ANNEXURES

ஒப்புதல் படிவம்

நோயாளியின் பெயர் :

வயது :

பாலினம் :

பெற்றோர் பெயர் :

முகவரி :

கோவை அரசு மருத்துவக் கல்லூரியில் நுண்ணுயிரியல் துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவர் மரு.கி. சிவராம் அவர்கள் மேற்கொள்ளும் எலும்பு முறிவுகளில் பொருத்தப்படும் இம்பிலாண்ட்களில் ஏற்படும் பாக்டீரியா தொற்று கிருமிகளை பற்றிய பரிசோதனை ஆய்வில் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களை தெளிவுபடுத்தி கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன். இந்த ஆய்வில் நான் முழுசம்மதத்துடன், சுய சிந்தனையுடன் கலந்து கொள்ள சம்மதிக்கிறேன். இந்த ஆய்வில் என்னைப்பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதலில் வெளியிடப்படுவதில் ஆட்சேபனையில்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்விலிருந்து நான் விலகிக் கொள்ள எனக்கு உரிமையுண்டு என்பதனையும் அறிவேன்.

இடம் கையொப்பம் / ரேகை

தேதி

CONSENT FORM

You, _____, aged ____ years, S/o / D/o / _____, residing at _____ _____ are requested to be a participant in the research study titled *"Isolation and Characterisation of Bacterial pathogens in Orthopaedic Implant Associated Infection in a Tertiary care hospital "* conducted by Dr.K.Sivaram, one of the post graduate trainees in the Dept. of Microbiology, Govt. Coimbatore Medical College and Hospital, Coimbatore. You are eligible for the study as per the inclusion criteria. You can ask him any question or seek from him any clarifications about the study which you may have before agreeing to participate in the study.

STATEMENT OF CONSENT

I, Father/Mother/Guardian of _____, do hereby volunteer and consent to my child participating in this study being conducted by Dr.K.Sivaram, I have read and understood the consent form (or) it has been read and explained to me thoroughly. I am fully aware of the study details as well as aware that I may ask questions to him at any time regarding this research on my ward.

Signature / Left Thumb Impression of the Father / Mother / Guardian

Station: Coimbatore

Date:

Signature / Left Thumb Impression and Name of the witness

Station: Coimbatore

Date:

MASTER CHART

S No	PATIENT NAME	AGE	SEX	PLACE	NATURE OF INJURY	ONSET OF INFECTION	TYPE OF FRACTURE	TYPE OF SURGERY	TYPE OF IMPLANT	ANTIBIOTIC PROPHYLAXIS	COMORBID /RISK FACTORS	CULTURE + /-	ORGANISM ISOLATED	GRAM+/-	DRUGS SENSITIVE	DRUGS RESISTANT	RESISTANT PATTERN
1	Nagaiya	53	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	GIVEN	2, 3 , 4,5,(	POSITIVE	PROTEUS SPP	NEGATIVE	AK, CIP, CFS	COT, AMX, GEN, CTX	
2	Chinnasamy	70	M	COIMBATORE	SELF FALL	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	NOT GIVEN	2, 3, 4,5,6	POSITIVE	E COLI	NEGATIVE	OF, CFS	COT, AMX CIP, GEN,AK,CTX	
3	Annapoorani	47	F	COIMBATORE	SELF FALL	ACUTE/EARLY	OPEN #	EMERGENCY	PLATE	NOT GIVEN	4, 6	POSITIVE	E COLI	NEGATIVE	CFS, PIT	COT, AMX, GEN, CTX,CIP,CN	
4	Vellusamy	60	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	DC PLATE/SCREW	GIVEN	4,5	NEGATIVE	NO GROWTH				
5	Bagyalakshmi	47	F	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	DC PLATE/SCREW	GIVEN	5,7	NEGATIVE	NO GROWTH				
6	Varadharaj	65	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	5,6	POSITIVE	S AUREUS	POSITIVE	AMX, CN, AK	COT, GEN, CIP, E	
7	Arumugam	70	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	EXT-FIXATOR	GIVEN	4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK, CFS	AMX, GEN, COT, CIP, CTX	
8	Masanan	65	M	NILGIRIS	Others	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3, 4	POSITIVE	PSEUDOMONAS	NEGATIVE	CIP, AK, CFS, CAZ	CN, AMX, GEN, CTX	
9	Sakthivel	39	M	NILGIRIS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	NEGATIVE	NO GROWTH				
10	Anandharaj	52	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	NEGATIVE	NO GROWTH				
11	Pattan	70	M	NILGIRIS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4	POSITIVE	PSEUDOMONAS	NEGATIVE	CN, AK, CTX, CFS	AMX, CIP	
12	Ramalingam	46	M	OTHERS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	NEGATIVE	NO GROWTH				
13	Muruganathan	50	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	CTX,CFS	COT, AMX, CN, CIP, AK, GEN	
14	Senthilkumar	27	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	PROSTHETIC DEVICE	GIVEN	7	POSITIVE	KLEBSIELLA SPP	NEGATIVE	COT, AMX, CIP, CN, GEN, AK,CFS	NIL	
15	Sathish	27	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	5,7	NEGATIVE	NO GROWTH				
16	Krishnamoorthy	50	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	EMERGENCY	DC PLATE/SCREW	NOT GIVEN	2,3	POSITIVE	CoNS	POSITIVE	E, DO, LZ, VAN	AMX, COT ,GEN	
17	Kannan	40	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	IM NAILING	NOT GIVEN	2	POSITIVE	POLYMICROBIAL				
18	Alagupandi	17	M	COIMBATORE	RTA	DELAYED	OPEN #	ELECTIVE	IM NAILING	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, GEN, CIP, LZ, VAN	COT, PEN, CN, E	
19	Ramamoorthy	40	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	EXT-FIXATOR	GIVEN	1,2,3,5	NEGATIVE	NO GROWTH				
20	Thannasi	58	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	GIVEN	1	POSITIVE	POLYMICROBIAL				
21	Kadar moidheen	54	M	TIRUPUR	RTA	DELAYED	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4	NEGATIVE	NO GROWTH				
22	Subramani	40	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4,5	POSITIVE	POLYMICROBIAL				
23	Srinivasan	46	M	COIMBATORE	Work spot in	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	NOT GIVEN	7	POSITIVE	PROTEUS SPP	NEGATIVE	AK, CFS	COT,AMX, CN, CIP, GEN, CTX	
24	Sathyaseelan	59	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	K-WIRE	NOT GIVEN	2,3,4	POSITIVE	PROTEUS SPP	NEGATIVE	GEN, AK,CTX, CFS	AMX, COT, CIP, CN	
25	Rajeshwari	45	F	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	POSITIVE	PROTEUS SPP	NEGATIVE	CIP, CFS,	AK, GEN, AMX, COT, CTX	
26	Natarajan	65	M	TIRUPUR	Self fall	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	2,5,6	POSITIVE	E COLI	NEGATIVE	AK	GEN CIP, CTX, AMX, COT	
27	Perumal	60	M	OTHERS	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	K-WIRE	NOT GIVEN	2,3	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK, CTX, CFS	GEN, AMX, COT,CIP, CN	
28	Teq bahadur	65	M	COIMBATORE	RTA	DELAYED	CLOSED #	ELECTIVE	K-WIRE	NOT GIVEN	2,3,4	POSITIVE	PROTEUS SPP	NEGATIVE	AK, CIP,	CTX, COT, AMX, CN,CAC	ESBL
29	Manoharan	65	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK,GEN,CIP	AMX, COT, CN,CTX,CAC	ESBL
30	Baby	43	F	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	GIVEN	4	POSITIVE	S AUREUS	POSITIVE	AMX, E, GEN, AK, CN,LZ,VAN	CIP,CTX, CX,CZ	MRSA
31	Kuppammal	48	F	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	POSITIVE	POLYMICROBIAL				
32	Puspha	46	F	COIMBATORE	SELF FALL	DELAYED	CLOSED #	ELECTIVE	PROSTHETIC DEVICE	NOT GIVEN	4,5	NEGATIVE	NO GROWTH				
33	Issaivananan	52	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	EXT-FIXATOR	GIVEN	6	POSITIVE	PROTEUS SPP	NEGATIVE	AK, GEN, CIP, MRP	CTX, CFS, COT	ESBL
34	Rengammal	70	F	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	GIVEN	4,5	POSITIVE	KLEBSIELLA SPP	NEGATIVE	GEN, AK, CIP, AMX,CTR	CTX, COT,CAC	ESBL
35	Mayakrishnan	19	M	TIRUPUR	SELF FALL	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	GIVEN	7	POSITIVE	PSEUDOMONAS	NEGATIVE	AK,,CIP, TOB	AMX, GEN, CN, CTX	
36	Raja	43	M	ERODE	Work spot in	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	GIVEN	7	NEGATIVE	NO GROWTH				
37	Akshay	26	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	NOT GIVEN	7	POSITIVE	PROTEUS SPP	NEGATIVE	AK. CTX	AMX, COT, CIP, CN, CFS	ESBL
38	Raman	55	M	OTHERS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	NOT GIVEN	4	POSITIVE	PSEUDOMONAS	NEGATIVE	GEN, AK,CIP	AMX, CN, CTX, DO,	
39	Thangavelu	63	M	TIRUPUR	SELF FALL	CHRONIC/LATE	CLOSED #	ELECTIVE	PROSTHETIC DEVICE	NOT GIVEN	1 ,4, 6	POSITIVE	ACINETOBACTER SPP	NEGATIVE	TOB, MRP	AMX, COT, CIP, CN,AK, CTX	
40	Senthil	27	M	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	IM NAILING	NOT GIVEN	7	POSITIVE	CoNS	POSITIVE	AMX, COT, GEN, AK	E, CN, CIP, CTX	
41	Muthukumar	48	M	COIMBATORE	RTA	CHRONIC/LATE	OPEN #	EMERGENCY	IM NAILING	GIVEN	7	POSITIVE	KLEBSIELLA SPP	NEGATIVE	GEN, AK, CIP, CN	CTX, CFS,AMC	
42	Ganesan	50	M	ERODE	Work spot in	ACUTE/EARLY	OPEN #	ELECTIVE	EXT-FIXATOR	GIVEN	2,3,4	POSITIVE	S AUREUS	POSITIVE	E, COT, DO, CN,CZ, LZ, VAN	GEN, AMX, CX	MRSA
43	Pitchai	61	M	TIRUPUR	Others	ACUTE/EARLY	OPEN #	ELECTIVE	PROSTHETIC DEVICE	GIVEN	2,4, 5,6	POSITIVE	E COLI	NEGATIVE	AK, GEN, CIP, CTX	AMX, CAC, CFS	ESBL
44	Arunthathadi	47	F	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	7	POSITIVE	PROTEUS SPP	NEGATIVE	PIT, MRP	GEN, AK, CTX CIP ,CFS	
45	Saraswathy	64	F	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	5,6	POSITIVE	S AUREUS	POSITIVE	GEN,AK, LZ,	AMX, CIP,CN, COT, DO	
46	Nagaraj	42	M	NILGIRIS	Others	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	GIVEN	7	POSITIVE	PROTEUS SPP	NEGATIVE	AMX, COY,GEN, AK, CIP,CTX,CFS	CN,	
47	Thulasiammal	80	F	COIMBATORE	SELF FALL	CHRONIC/LATE	CLOSED #	ELECTIVE	PLATE	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, COT, GEN, AK,CZ,LZ, VAN	E, CN,CX	MRSA
48	Thangammal	60	F	TIRUPUR	RTA	DELAYED	OPEN #	ELECTIVE	IM NAILING	GIVEN	1,4,6	POSITIVE	POLYMICROBIAL				
49	Palanisamy	45	M	TIRUPUR	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	GIVEN	2,4	NEGATIVE	NO GROWTH				
50	Suresh	29	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	K-WIRE	GIVEN	4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK, CFS	AMX, GEN,CIP, CTX,CEC	ESBL
51	Chinnasamy	42	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	GIVEN	2,3, 4	POSITIVE	S AUREUS	POSITIVE	GEN, AK, CIP, LZ	E, AMX, COT,DO	
52	Ajith kumar	19	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	GIVEN	5,7	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK,CIP,GEN	AMX,COT,CN,CTX, CFS	ESBL
53	Ayyasamy	41	M	NILGIRIS	RTA	DELAYED	OPEN #	EMERGENCY	IM NAILING	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	LZ CFS, VAN	E,DO,AMX, GEN, AK, CIP	
54	Jayakumar	50	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	IM NAILING	GIVEN	7	POSITIVE	PSEUDOMONAS	NEGATIVE	TOB, CAZ	GEN, AK, CIP, CN, CTX	
55	Rajendran	25	M	COIMBATORE	SELF FALL	ACUTE/EARLY	OPEN #	EMERGENCY	PLATE	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, GEN, AK,CN, LZ,VAN	E,COT, CX,CZ	MRSA
56	Thulasiammal	85	F	TIRUPUR	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	DC PLATE/SCREW	NOT GIVEN	4,6	POSITIVE	S AUREUS	POSITIVE	E, GEN, AK, CN, LZ,VAN	AMX, COT,CIP	
57	Govindhasamy	68	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	DC PLATE/SCREW	GIVEN	2,3,4,6	POSITIVE	ACINETOBACTER SPP	NEGATIVE	TOB.MRP	AK, GEN, CIP,CTX,	
58	Muthukumar	23	M	NILGIRIS	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, CN,CN, GEN ,LZ, VAN	COT, E,CX	MRSA
59	Mani	40	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	PROSTHETIC DEVICE	GIVEN	2,3	POSITIVE	S AUREUS	POSITIVE	AK, LZ,VAN	E, AMX, COT,DO,CIP	

60	Ramasamy	55	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	2,3	POSITIVE	E COLI	NEGATIVE	AK, CIP, CFS	AMX, COT, GEN, CN, CTX	
61	Aravindhan	18	M	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	PLATE	GIVEN	5,7	NO GROWTH	NO GROWTH				
62	Jeyaraj	45	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	IM NAILING	NOT GIVEN	2,3,4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	CIP,AK,AMX	CN, GEN, CTX, COT,CAZ	ESBL
63	Jeeva	7	Mc	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	IM NAILING	NOT GIVEN	7	NEGATIVE	NO GROWTH				
64	Logeshwaran	8	Mc	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	K-WIRE	NOT GIVEN	7	NEGATIVE	NO GROWTH				
65	Raja	61	m	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4	POSITIVE	PROTEUS SPP	NEGATIVE	AK, CFS	AMX, COT, GEN, CTX, CAC	ESBL
66	Pushpalatha	48	F	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PROSTHETIC DEVICE	NOT GIVEN	4	POSITIVE	ENTEROCOCCUS	POSITIVE	CIP, AMC, CD,VAN	E, AMX, DO, HLG, COT	
67	Eswaran	57	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	3,3,	POSITIVE	PSEUDOMONAS	NEGATIVE	AK, CFS	AMX, GEN, COT, CIP, CTX	
68	Manikandan	18	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AK, LZ, CX,VAN	E,AMX, DO, CN,GEN	
69	Kavitha	35	F	NILGIRIS	RTA	DELAYED	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	NO GROWTH	NO GROWTH				
70	Rajammal	60	F	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	GIVEN	1,4	POSITIVE	S AUREUS	POSITIVE	E, CD, CX, LZ,VAN, AMC	AMX, GEN, CIP, CN	
71	Selvi	56	F	OTHERS	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	IM NAILING	GIVEN	1,4,6	POSITIVE	S AUREUS	POSITIVE	AMX, GEN, AK, CN,CX,VAN	E, COT, CIP,	
72	Ithiga	5	Fch	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	K-WIRE	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, DO, E, CD, CX, LZ, VAN	COT, PEN, GEN	
73	Parija	28	F	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	NOT GIVEN	4	POSITIVE	S AUREUS	POSITIVE	CX,LZ,VAN	AMX, COT, GEN, CIP, CN,CTX	
74	Pappathi	65	F	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	4,6	POSITIVE	S AUREUS	POSITIVE	E, DO, CN,CX, LZ, VAN	AMX, COT, GEN	
75	Jakkamal	65	F	TIRUPUR	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PROSTHETIC DEVICE	NOT GIVEN	4	POSITIVE	S AUREUS	POSITIVE	AK, LZ,CX,VAN	AMX, COT, GEN, CN, CIP,CTX	
76	Kaviya	7	Fch	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	IM NAILING	NOT GIVEN	7	POSITIVE	CoNS	POSITIVE	AMX,COT,GEN, AK	E, CN, CIP, CTX	
77	Sasikumar	16	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	PLATE	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AK, LZ, CX,VAN	E, AMX ,GEN, CN, DO	
78	Chandran	42	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	NOT GIVEN	2	POSITIVE	E COLI	NEGATIVE	GEN, AK, CIP, AMC,CTR	CTX, CTR, COT,CAZ	ESBL
79	Yogeshwaran	38	M	ERODE	SELF FALL	CHRONIC/LATE	CLOSED #	ELECTIVE	PROSTHETIC DEVICE	GIVEN	2,4,5	POSITIVE	POLYMICROBIAL				
80	Ayyasamy	41	M	NILGIRIS	RTA	DELAYED	OPEN #	ELECTIVE	IM NAILING	GIVEN	5,7	NEGATIVE	NO GROWTH				
81	Thangammal	65	F	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	NOT GIVEN	4	POSITIVE	S AUREUS	POSITIVE	AMX, GEN, CD, DO, CX,LZ, VAN	PEN, COT, E, CIP, CN	
82	Manikandan	18	M	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	PLATE	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	CX. LZ, VAN	E, AMX, DO, GEN, AK, CN	
83	Rajendran	57	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	K-WIRE	NOT GIVEN	2,3,5,6	POSITIVE	S AUREUS	POSITIVE	E, COT, CN, CD, LZ,CZ, VAN	GEN, CX,CIP	MRSA
84	Arumugam	65	M	COIMBATORE	SELF FALL	CHRONIC/LATE	CLOSED #	ELECTIVE	PLATE	NOT GIVEN	2,3	NEGATIVE	NO GROWTH				
85	Prabu	50	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	GIVEN	4,5	POSITIVE	CoNS	POSITIVE	CX, LZ, VAN	E, DO, CIP, CN, GEN	
86	Jailani	23	M	TIRUPUR	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	K-WIRE	NOT GIVEN	7	NEGATIVE	NO GROWTH				
87	Sekar	36	M	COIMBATORE	RTA	ACUTE/EARLY	CLOSED #	ELECTIVE	IM NAILING	NOT GIVEN	5,7	POSITIVE	S AUREUS	POSITIVE	AMX, AK, CX, LZ, VAN	COT, CIP, DO, GEN, CN	
88	Krishnakumar	23	M	NILGIRIS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	POSITIVE	PSEUDOMONAS	NEGATIVE	AK, TOB, CAZ	CIP, GEN, CTX	
89	Aruchamy	32	M	COIMBATORE	SELF FALL	DELAYED	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	1,6	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK, CFS	AMX, GEN, CIP, CTX	
90	Selvaraj	35	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	GIVEN	7	POSITIVE	PSEUDOMONAS	NEGATIVE	GEN, AK, TOB, PIT	CIP, CAZ, CTX	
91	Thangammal	60	F	OTHERS	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	DC PLATE/SCREW	NOT GIVEN	4	POSITIVE	PROTEUS SPP	NEGATIVE	AK, CTX	GEN, AMX, CIP, CFS	ESBL
92	Nivetha	17	F	COIMBATORE	Others	DELAYED	CLOSED #	ELECTIVE	IM NAILING	NOT GIVEN	7	NEGATIVE	NO GROWTH				
93	Pothiappan	41	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	NEGATIVE	NO GROWTH				
94	Ponnusamy	55	M	COIMBATORE	RTA	DELAYED	OPEN #	ELECTIVE	IM NAILING	GIVEN	1,2	NEGATIVE	NO GROWTH				
95	Chinnasamy	60	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3,4,6	POSITIVE	PROTEUS SPP	NEGATIVE	AK, PIT, MRP,CTR	GEN, CIP, AMC, CTX, CFS	ESBL
96	Jeganathan	60	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	NOT GIVEN	2,3,4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	OF, PIT, MRP,CTX	GEN, AK, CIP, CFS, CEC	ESBL
97	Rangammal	45	F	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	POSITIVE	ACINETOBACTER SPP	NEGATIVE	AK,PIT,CFS	GEN,CIP, CTX, CAZ,	
98	Bannari	46	F	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	NOT GIVEN	4	POSITIVE	PSEUDOMONAS	NEGATIVE	GEN, AK, , PIT, CPM, MRP,CAZ	CIP, LE	
99	Anbumani	30	M	COIMBATORE	Assault	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	NO GROWTH	NO GROWTH				
100	Kannan	40	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,4,5	POSITIVE	E COLI	NEGATIVE	AMX, CIP, CN, COT, CTX	GEN, AK,CFS	ESBL
101	Vellingiri	35	M	COIMBATORE	SELF FALL	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	NOT GIVEN	4,5	POSITIVE	E COLI	NEGATIVE	AK,CIP,	AMX, COT, CTX, CFS,GEN	ESBL
102	Perumal	60	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	GIVEN	2,3,	NEGATIVE	NO GROWTH				
103	Sasikumar	27	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	IM NAILING	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX,E, DO, CN, GEN, AK, CX	COT,CIP,AK	
104	Akshay	20	M	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK,CIP	GEN, CTX, CFS	ESBL
105	Karuppusamy	25	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	7	GIVEN	7	POSITIVE	S AUREUS	POSITIVE	E,COT, CD, DO,CN, LZ, VAN	AMX, GEN, CX ,CZ	MRSA
106	Lakshmi	62	F	ERODE	SELF FALL	CHRONIC/LATE	CLOSED #	ELECTIVE	IM NAILING	GIVEN	4,5	POSITIVE	KLEBSIELLA SPP	NEGATIVE	COT, GEN, AK, CIP,CTR	AMX, CN, CTX,CFS	ESBL
107	Sharaj	42	F	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	IM NAILING	GIVEN	1,3,4,5	NO GROWTH	NO GROWTH				
108	Velmurugan	27	M	COIMBATORE	Work spot in	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	6	POSITIVE	KLEBSIELLA SPP	NEGATIVE	AK,CFS,PIT, MRP	AMX, GEN, CIP, OF	
109	Senthilkumar	42	M	COIMBATORE	RTA	DELAYED	CLOSED #	EMERGENCY	K-WIRE	NOT GIVEN	2,3	POSITIVE	S AUREUS	POSITIVE	E, DO, CIP, LZ, VAN,CZ	COT, GEN ,PEN, CX	MRSA
110	Manoj	42	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,4	POSITIVE	PSEUDOMONAS	NEGATIVE	GEN,AK, CIP, CTX, CAZ	AMX, CN,CFS	
111	Senthikumar	33	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	2,3	POSITIVE	KLEBSIELLA SPP	NEGATIVE	CIP, CTX,CN,AK	AMX,COT, GEN, CFS,CEC	ESBL
112	Oviyammal	75	F	NILGIRIS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	POSITIVE	PROTEUS SPP	NEGATIVE	AK, CIP, CFS	AMX,COT, GEN, CN, CTX	
113	Dhakshinamurthy	54	M	NILGIRIS	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	K-WIRE	GIVEN	1,2,3	POSITIVE	KLEBSIELLA SPP	NEGATIVE	CIP, AK, CAZ	AMX, COT, GEN, CN, CTX	
114	Sivaraj	37	M	COIMBATORE	SELF FALL	ACUTE/EARLY	CLOSED #	ELECTIVE	PLATE	GIVEN	5,7	POSITIVE	KLEBSIELLA SPP	NEGATIVE	GN, AK, CIP, CTR, AMX, COT	CTX, CFS	ESBL
115	Vignesh	19	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	POSITIVE	PSEUDOMONAS	NEGATIVE	GEN, AK, CIP CAZ	AMX, CN, CTX	
116	Ranjith kumar	32	M	COIMBATORE	RTA	CHRONIC/LATE	CLOSED #	ELECTIVE	EXT-FIXATOR	GIVEN	7	POSITIVE	PSEUDOMONAS	NEGATIVE	, GEN, AK, CAZ,TOB, CPM	CIP, LE, CTX	
117	Navaneethakrishnan	23	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	POSITIVE	S AUREUS	POSITIVE	E, GEN, CN, LZ, VAN,	AMX, CX, CIP,CZ	MRSA
118	Karnan	45	M	COIMBATORE	SELF FALL	ACUTE/EARLY	OPEN #	ELECTIVE	PROSTHETIC DEVICE	NOT GIVEN	2,3	POSITIVE	CoNS	POSITIVE	AMX, COT, GEN, AK	E, CIP, CN, CTX	
119	Bhagavathy	75	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	NOT GIVEN	2	POSITIVE	PSEUDOMONAS	NEGATIVE	GN, AK, CIP, CFS,CAZ, TOB	AMX, CN, CTX	
120	Mothila	25	M	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	4	POSITIVE	PROTEUS SPP	NEGATIVE	CIP,CFS	AMX, COT,GN, AK, CN,CTX	
121	Kanniyappan	37	M	TIRUPUR	RTA	CHRONIC/LATE	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	7	POSITIVE	KLEBSIELLA SPP	NEGATIVE	CIP, AK, CAZ	AMX, COT, GN, CN,CTX	
122	Riyas	25	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	PROSTHETIC DEVICE	GIVEN	4,5	POSITIVE	POLYMICROBIAL				
123	Sharaj	40	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	PLATE	GIVEN	2,4	POSITIVE	S AUREUS	POSITIVE	E, GEN, CIP, CN,CD, DO, LZ, VAN	AMX, COT, PEN	
124	Muralidharan	38	M	COIMBATORE	RTA	CHRONIC/LATE	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	1	POSITIVE	CoNS	POSITIVE	AMX, COT, GEN, AK, CN, CTX	E, CIP,	
125	Gopal	42	M	COIMBATORE	RTA	CHRONIC/LATE	OPEN #	EMERGENCY	IM NAILING	NOT GIVEN	2,3,5	POSITIVE	KLEBSIELLA SPP	NEGATIVE	CTX, PIT,MRP	GEN CIP, AMX, COT,CEC,	ESBL
126	Thillaiammal	55	F	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	GIVEN	1,4	POSITIVE	KLEBSIELLA SPP	NEGATIVE	COT,AK, GEN,CIP	AMX, CN, CTX, CFS	ESBL

127	Kannamal	65	F	COIMBATORE	RTA	DELAYED	OPEN #	EMERGENCY	PROSTHETIC DEVICE	GIVEN	4	POSITIVE	PSEUDOMONAS	NEGATIVE	AK, TOB, CTX, CIP, CAZ	AMX, COT, GEN	
128	Lakshmi	62	F	ERODE	SELF FALL	CHRONIC/LATE	CLOSED #	ELECTIVE	PROSTHETIC DEVICE	GIVEN	1,4,5,6	POSITIVE	CoNS	POSITIVE	AMX, GEN, CN, E, AK, CTX	COT, CIP, DO	
129	Arunagiri	45	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	PROSTHETIC DEVICE	GIVEN	2,4,5	POSITIVE	S AUREUS	POSITIVE	E, AK, CN, GEN, LZ, VAN,CZ	AMX, COT, CIP, CX	MRSA
130	Mani	53	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	GIVEN	1,2,6	NEGATIVE	NO GROWTH				
131	Vijayakumar	24	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	NOT GIVEN	7	POSITIVE	CoNS	POSITIVE	AMX, CD, CX, LZ, VAN	E, DO, CIP, CN, GEN	
132	Sanjay	14	M	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	PLATE	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, AK, LZ, CD, VAN	E, DO, GEN, COT, CN, CTX	
133	Nandhini	24	F	TIRUPUR	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	EXT-FIXATOR	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	E, DO, AMX, GEN, CX, LZ,VAN	COT, CIP	
134	Varadharajan	61	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	ELECTIVE	PLATE	NOT GIVEN	1,4,6	POSITIVE	S AUREUS	POSITIVE	AMX, AK, CX,LZ, VAN	E, DO,CN, CIP, COT, CTX	
135	Lawrance	50	M	ERODE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	IM NAILING	GIVEN	2,	NO GROWTH	NO GROWTH				
136	Mahalakshmi	32	F	TIRUPUR	SELF FALL	ACUTE/EARLY	CLOSED #	EMERGENCY	PLATE	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	AMX, DO, CN, GEN, AK, CTX	E, COT, CIP,	
137	Madhan	27	M	COIMBATORE	RTA	ACUTE/EARLY	OPEN #	EMERGENCY	PLATE	NOT GIVEN	7	POSITIVE	S AUREUS	POSITIVE	E,AMX, DO, CN, LZ, VAN,	CIP, CX,CZ	MRSA

M: MALE
F: FEMALE
RTA: ROAD TRAFFIC ACCIDENT
1: Diabetes Mellitus
2: Smoking
3: Alcoholism
4: Haemoglobin level <12gm/dl
5: Total count >12000/cumm
6: SHT/CKD/CLD/EPILEPSY etc
7: No risk factors